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(54) **IN SITU ANTIGEN-GENERATING CANCER VACCINE**

(71) Applicant: **President and Fellows of Harvard College, Cambridge, MA (US)**

(72) Inventors: **Jaeyun Kim, Gyeonggi-do (KR); David J. Mooney, Sudbury, MA (US); Weiwei Aileen Li, Norcross, GA (US); Praveen Arany, Cambridge, MA (US); Or Gadish, Cambridge, MA (US)**

(73) Assignee: **President and Fellows of Harvard College, Cambridge, MA (US)**

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(58) **Field of Classification Search**

None
See application file for complete search history.

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Primary Examiner — Sean E Aeder

(74) *Attorney, Agent, or Firm* — McCarter & English, LLP; Maria Laccotripe Zacharakis; Maneesh Gulati

(57) **ABSTRACT**

The invention provides compositions and methods for utilizing scaffolds in cancer vaccines.

38 Claims, 21 Drawing Sheets

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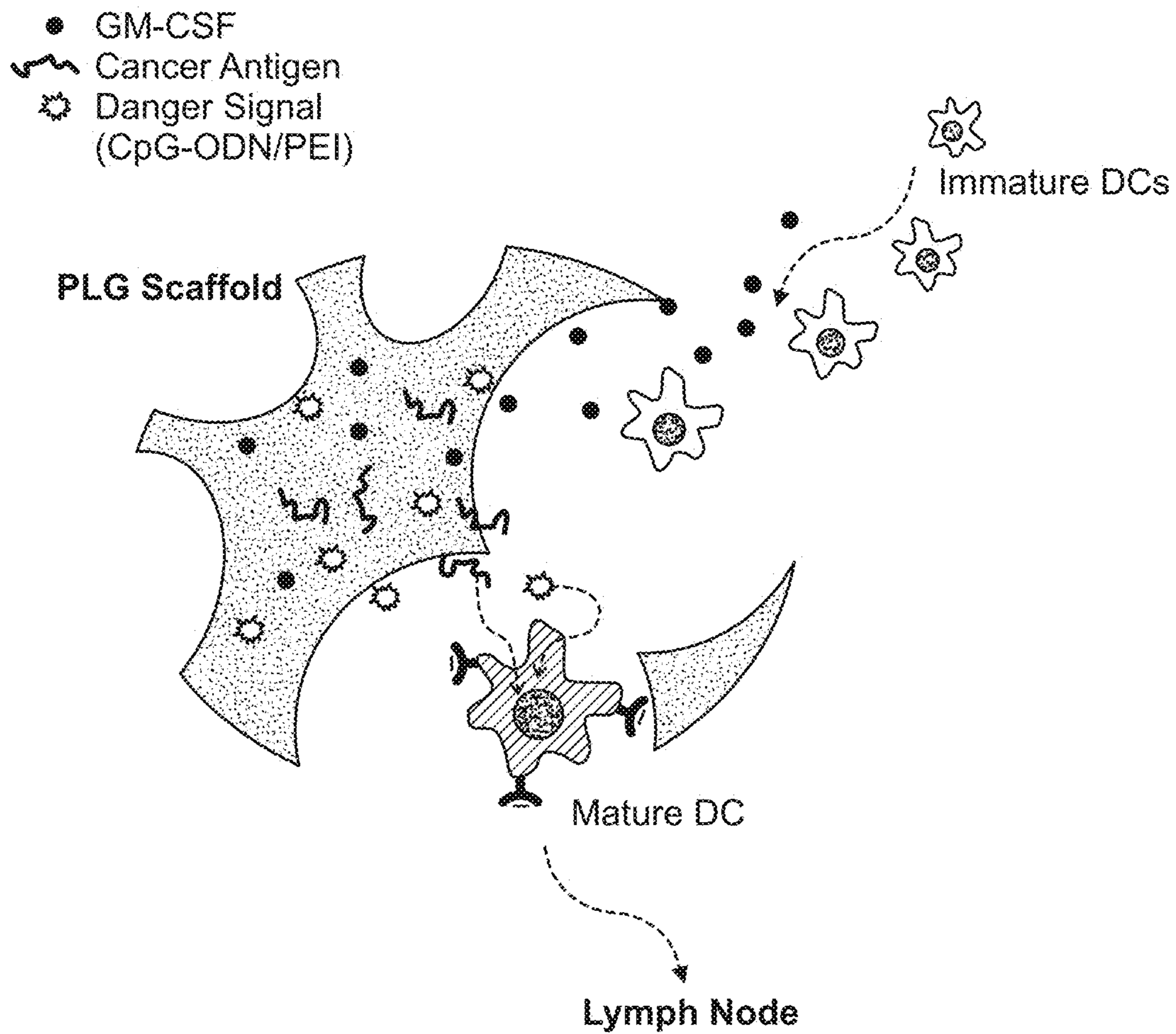


FIG. 1

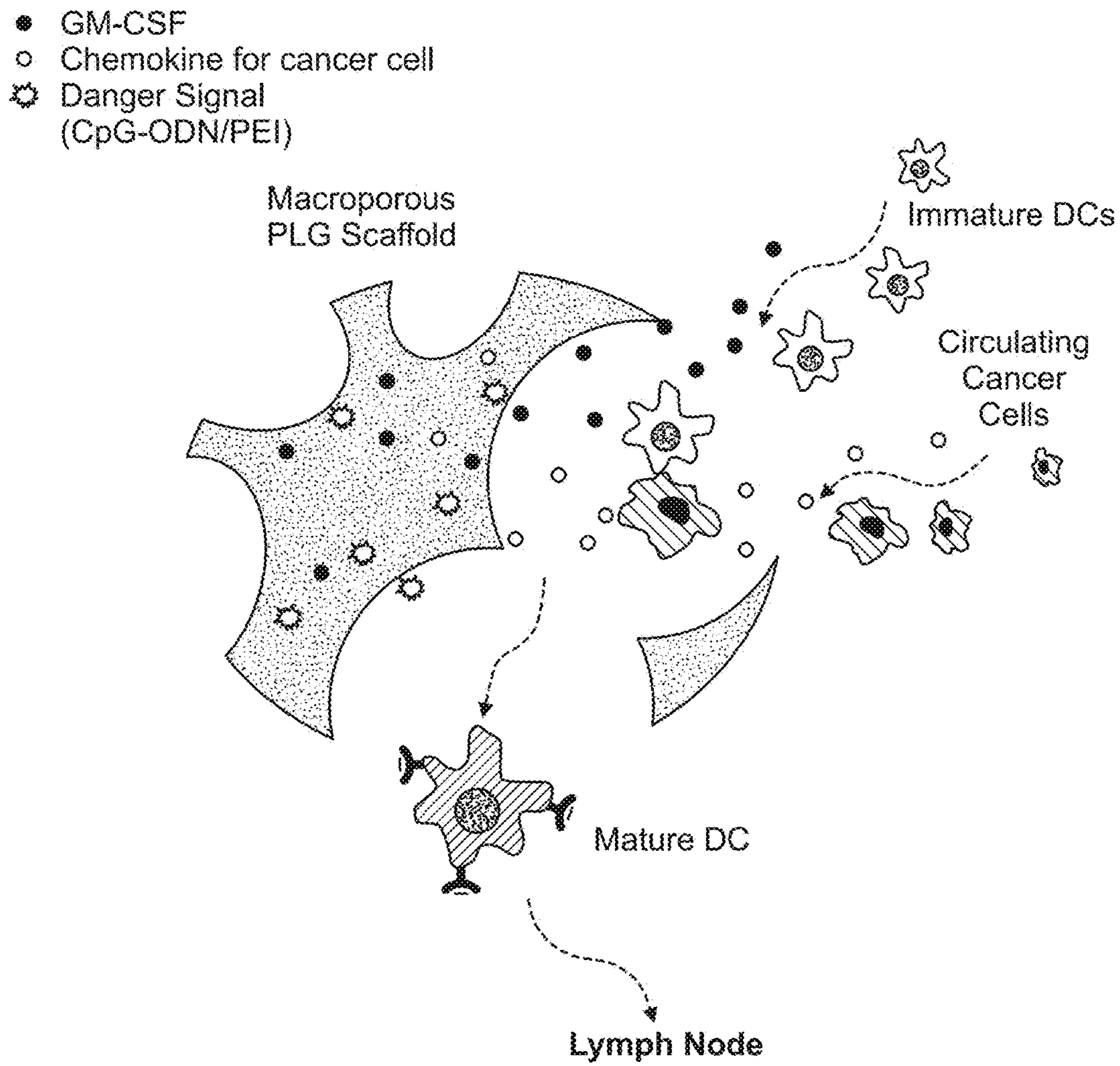


FIG. 2

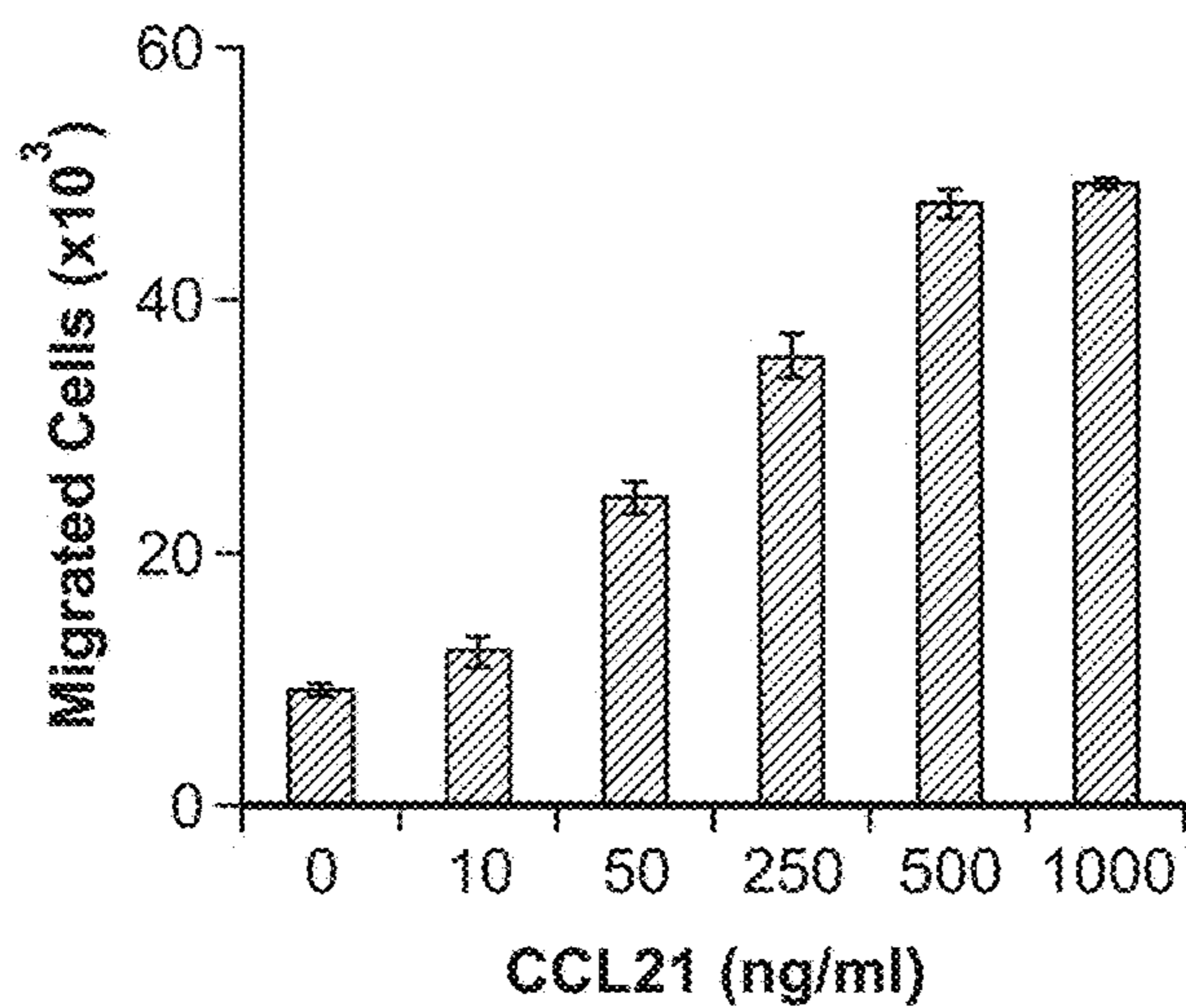


FIG. 3

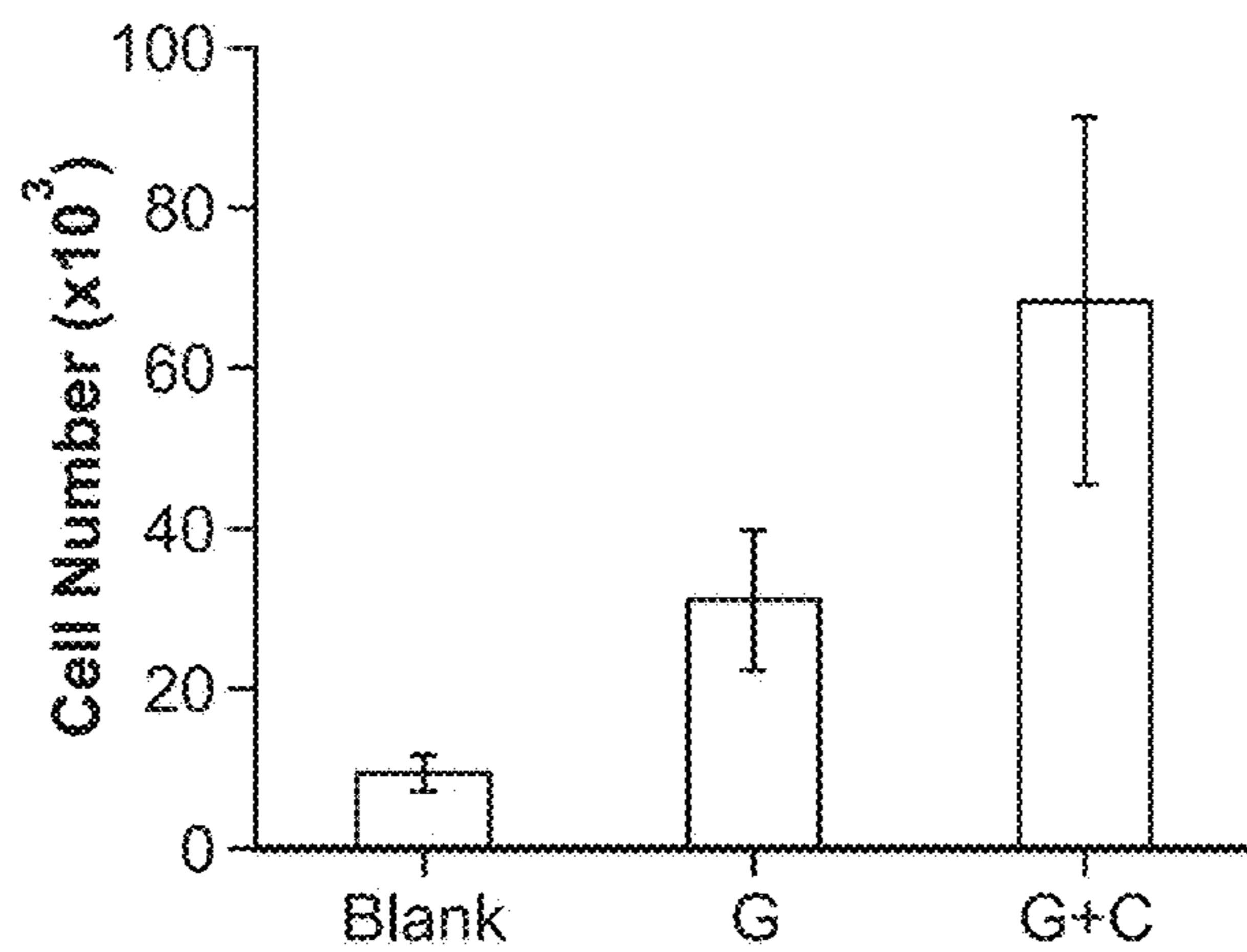


FIG. 4

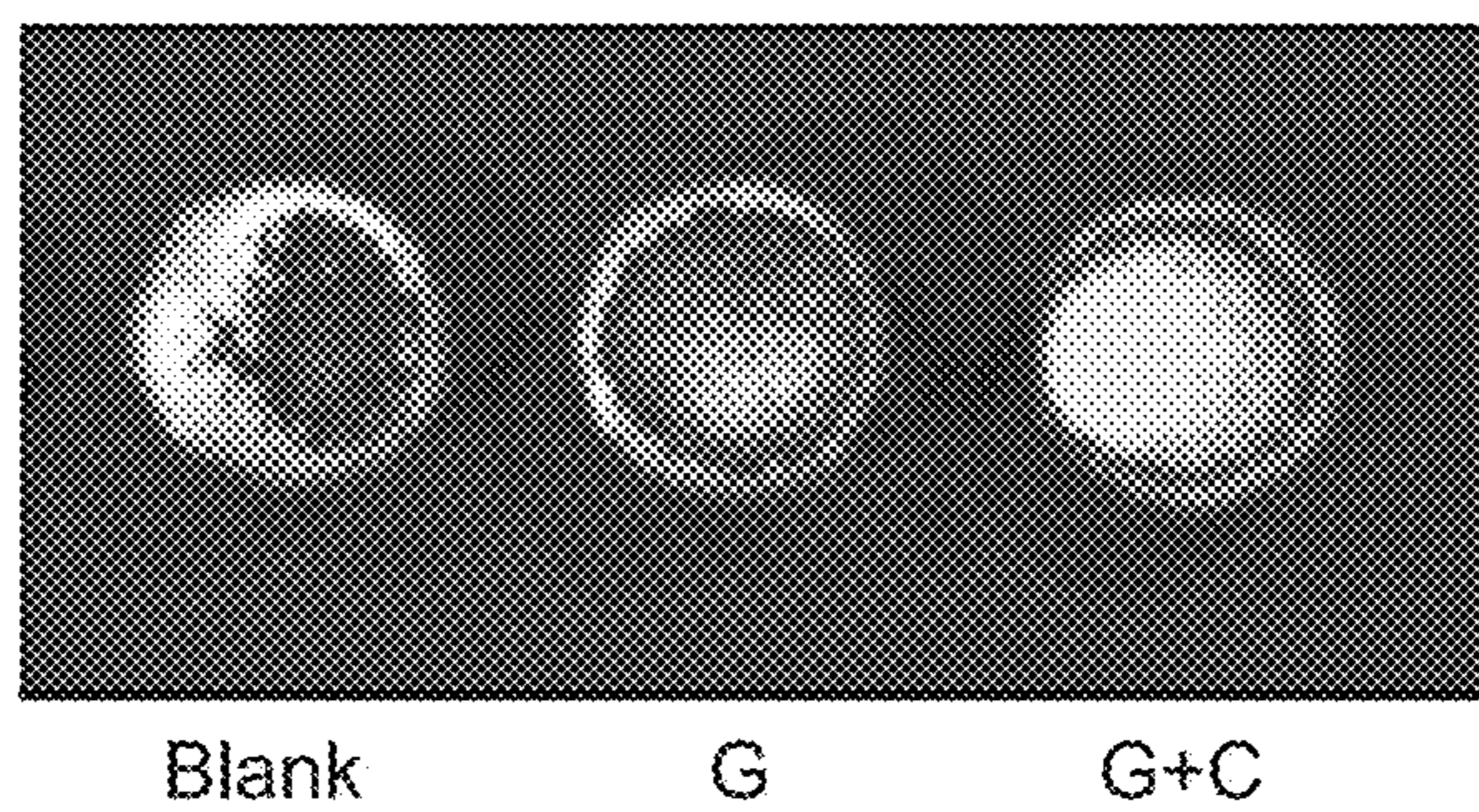


FIG. 5

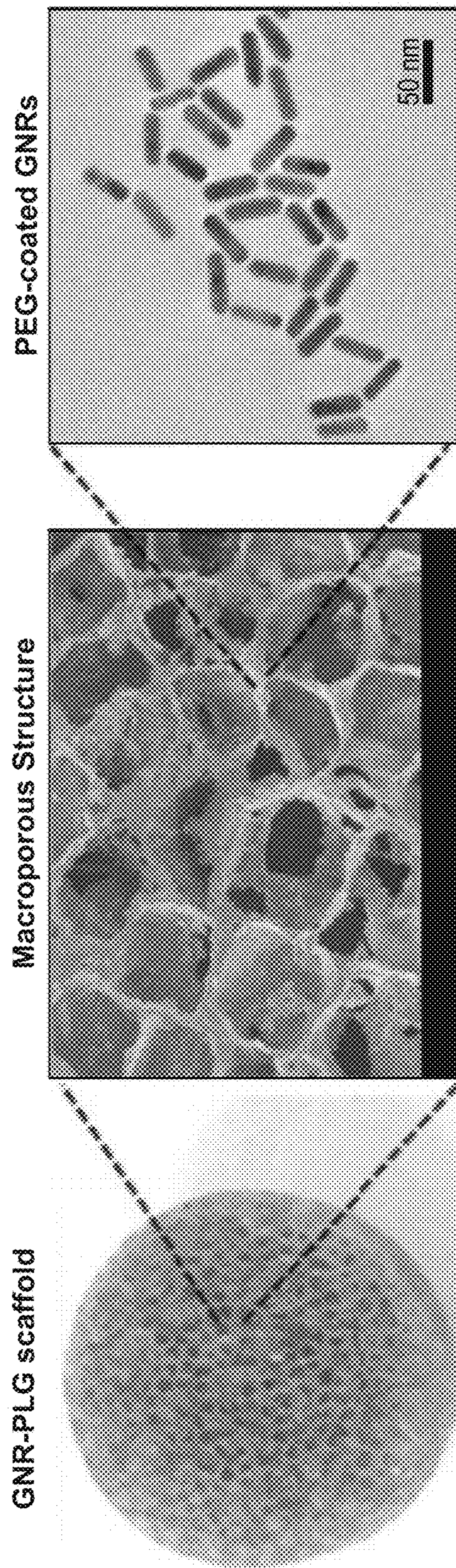


FIG. 6C

FIG. 6B

FIG. 6A

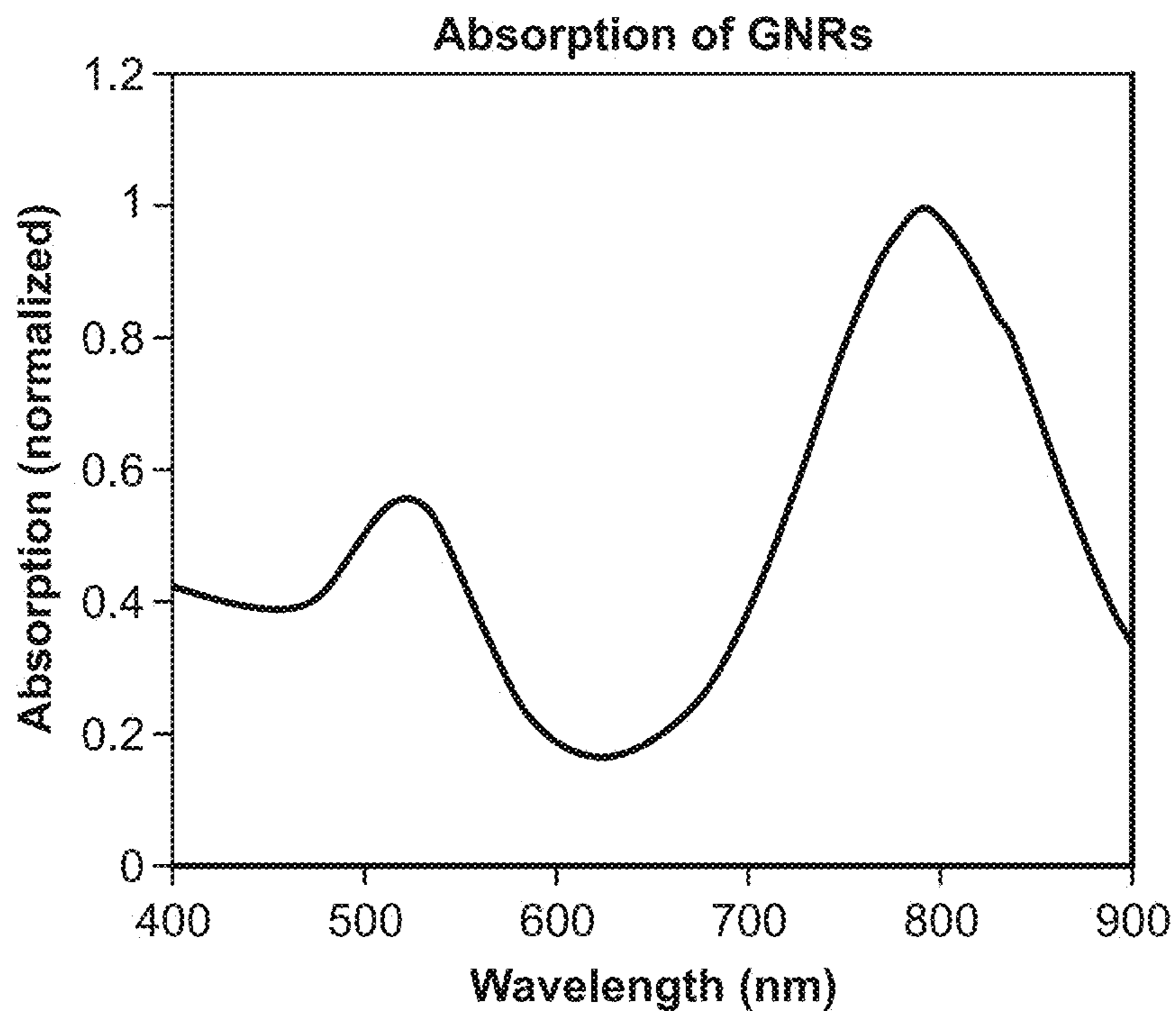


FIG. 7A

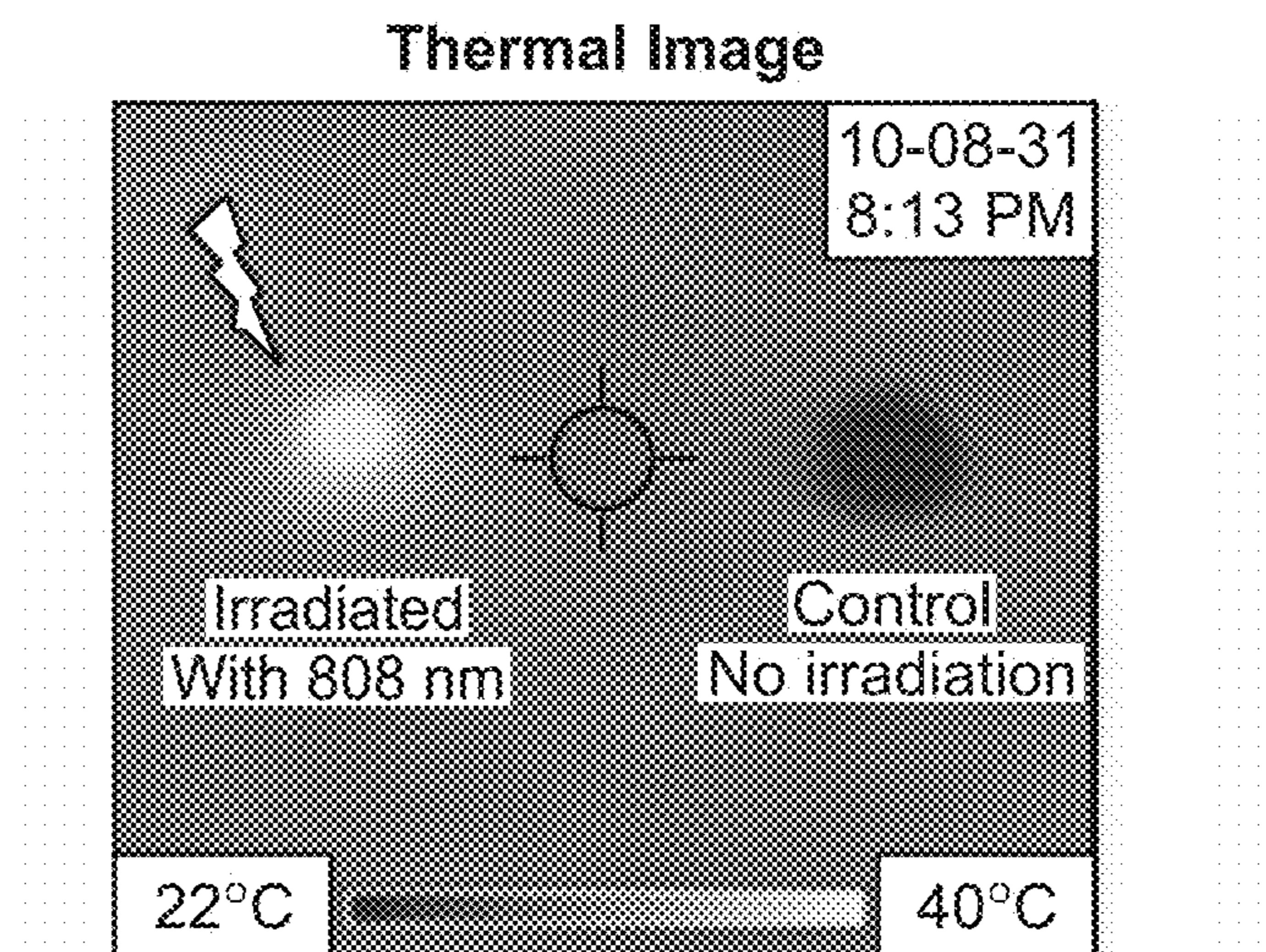


FIG. 7B

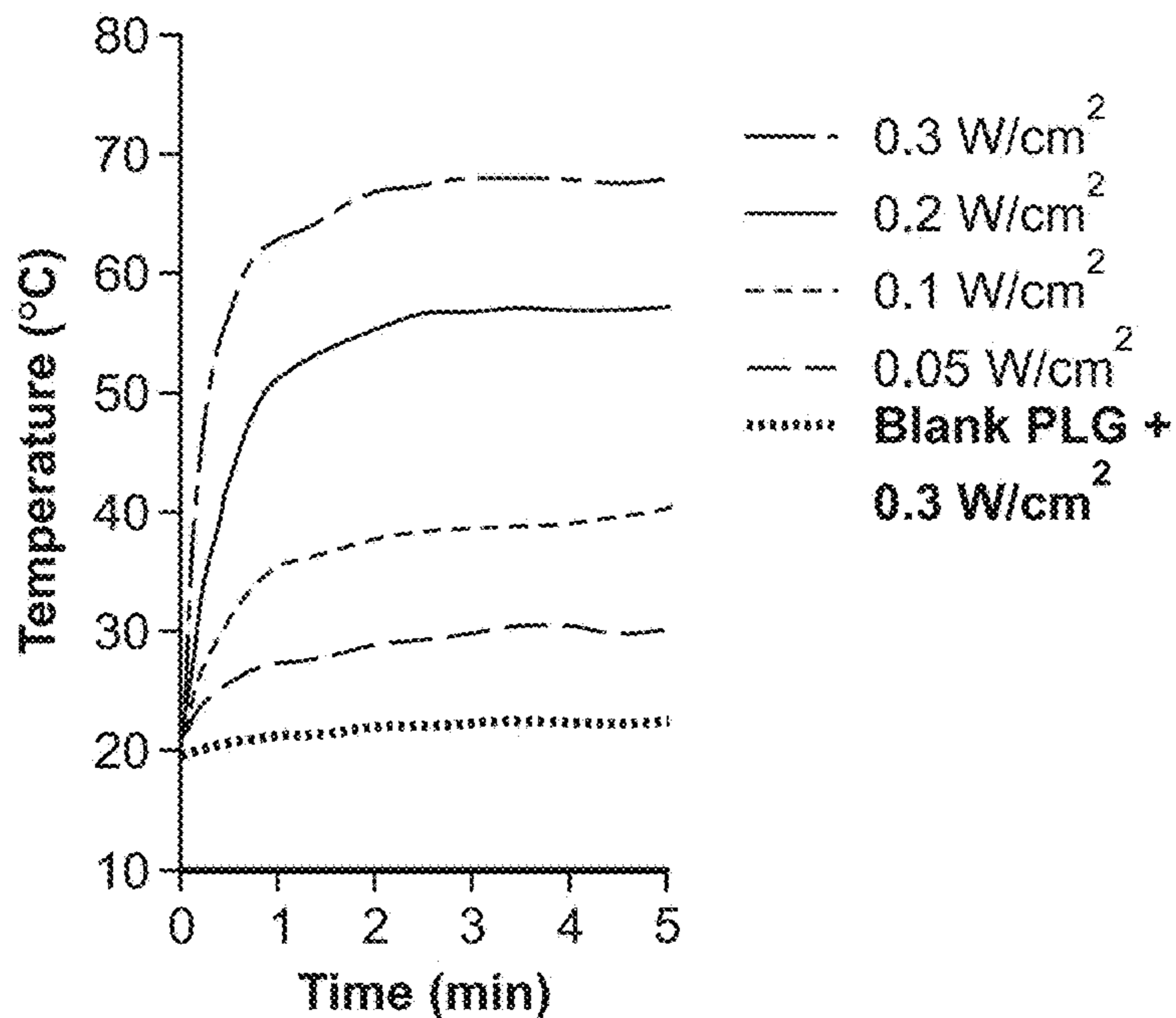


FIG. 8A

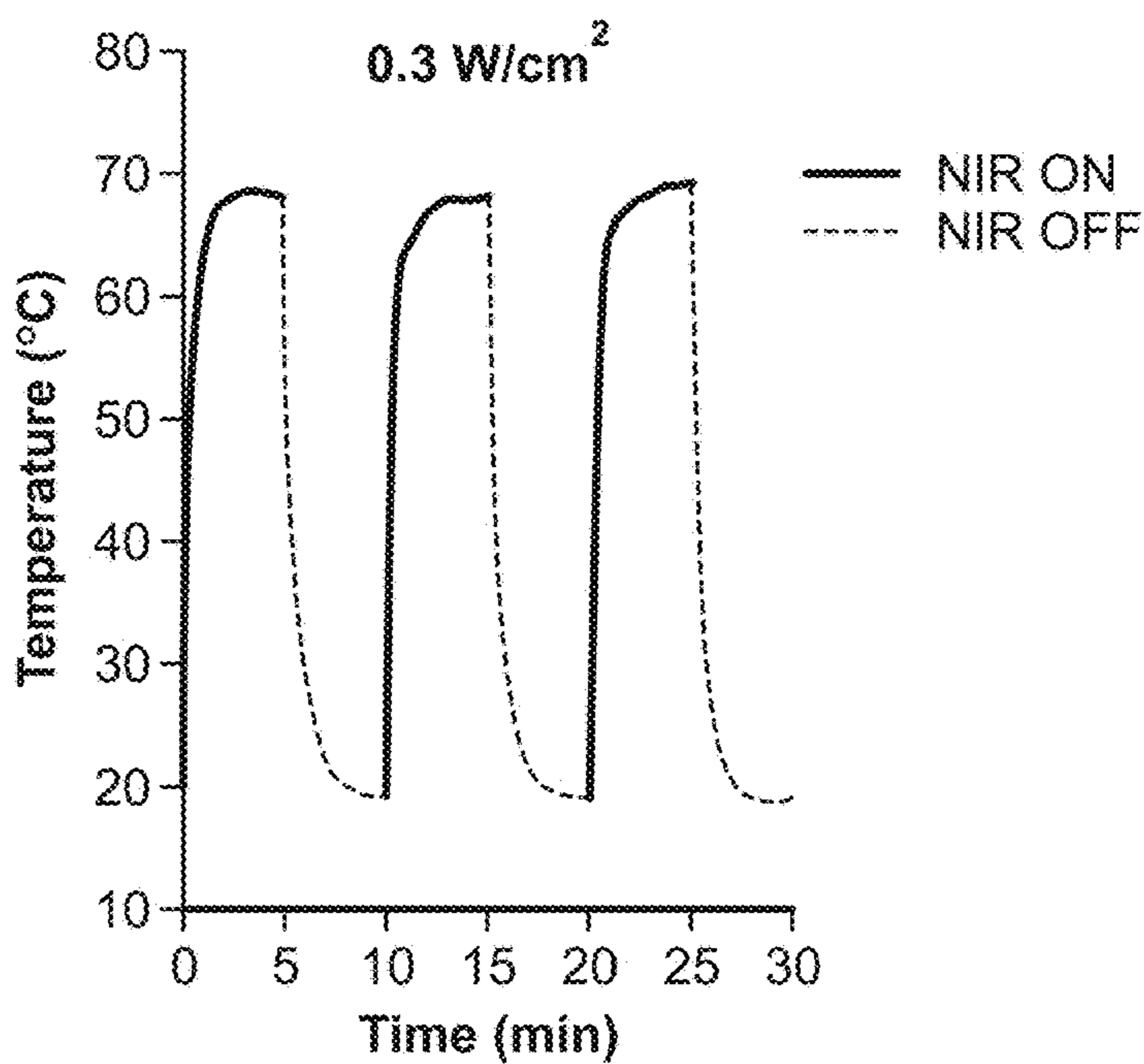


FIG. 8B

FIG. 9

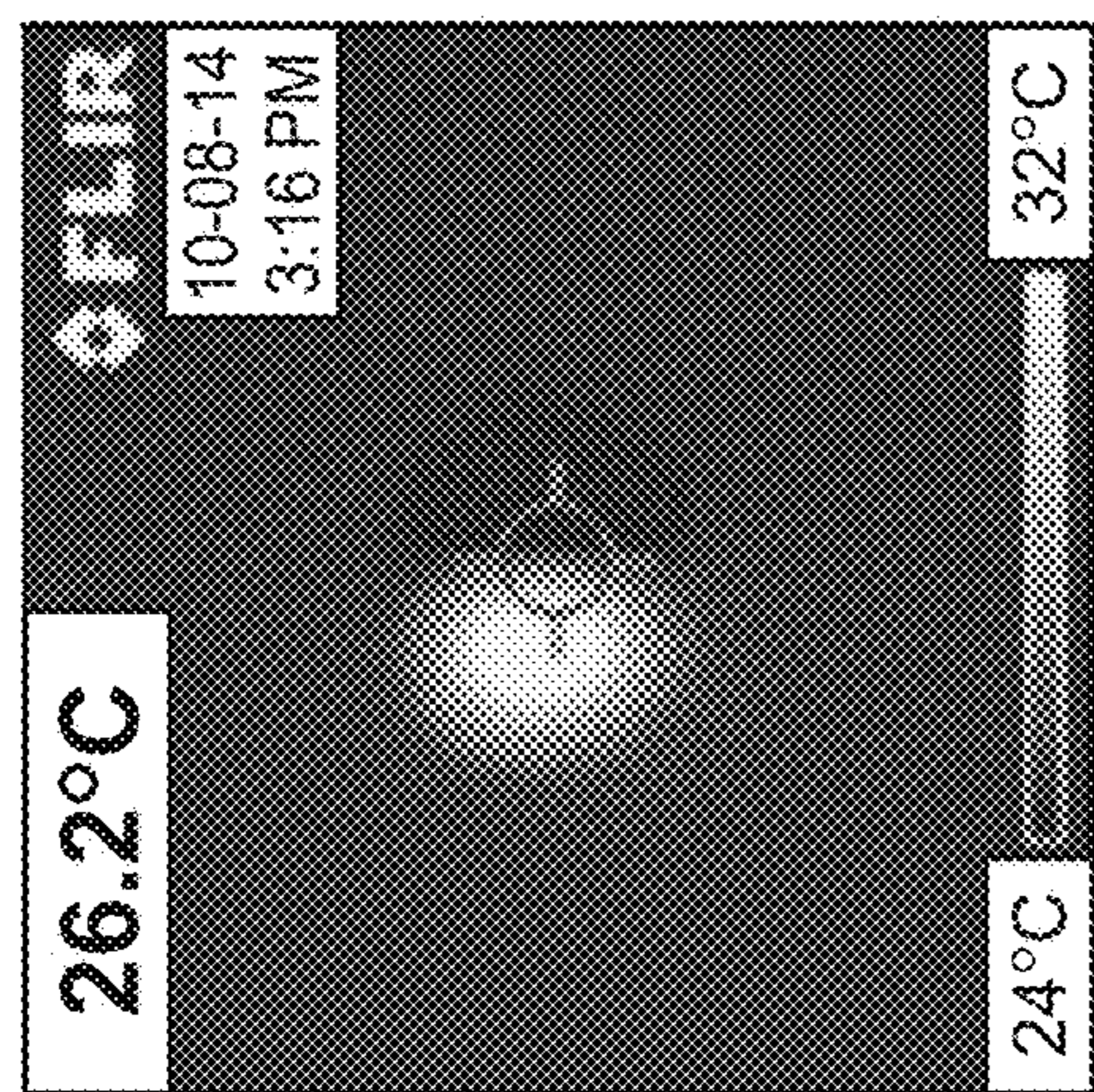


FIG. 10B

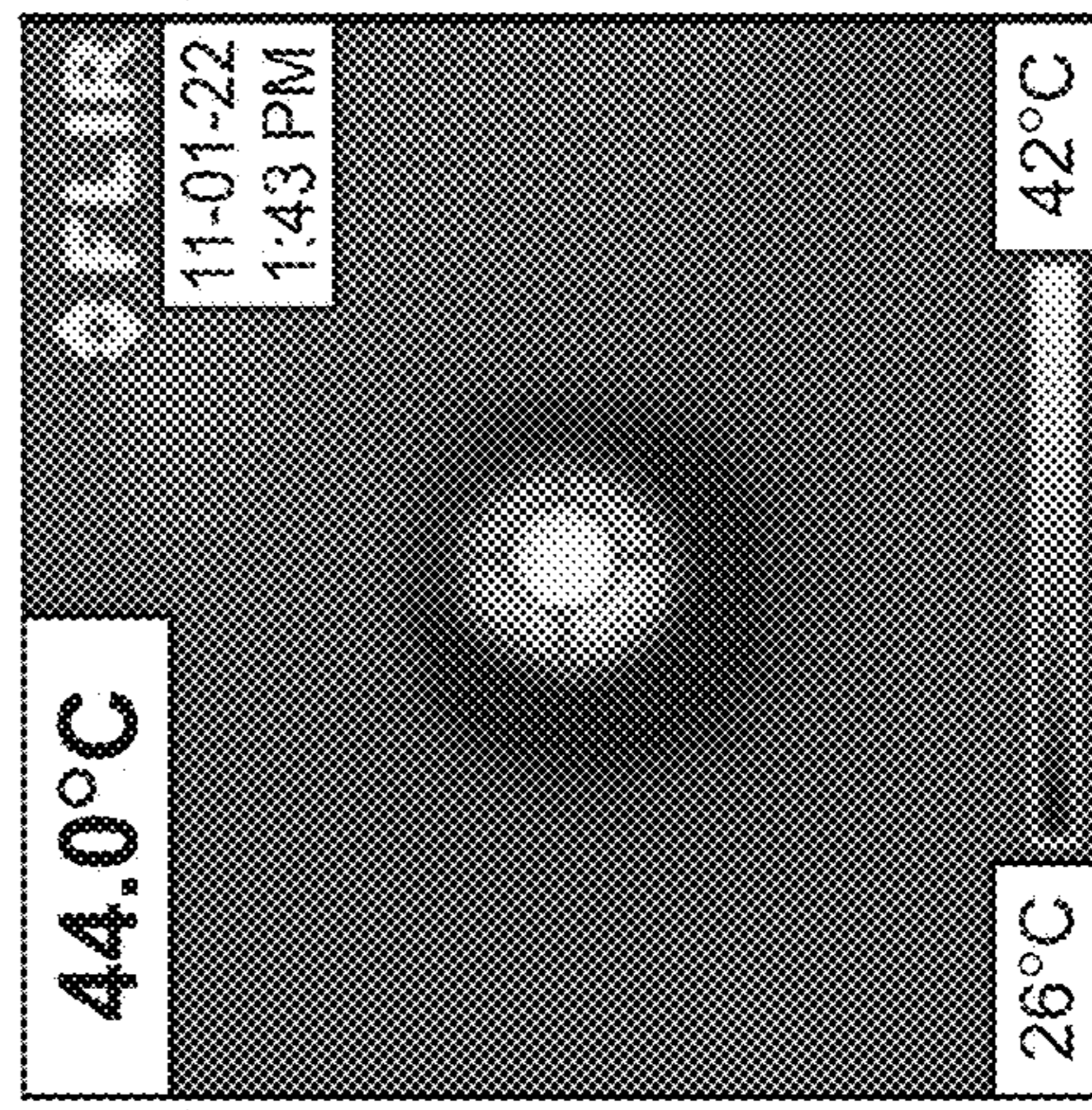


FIG. 10C

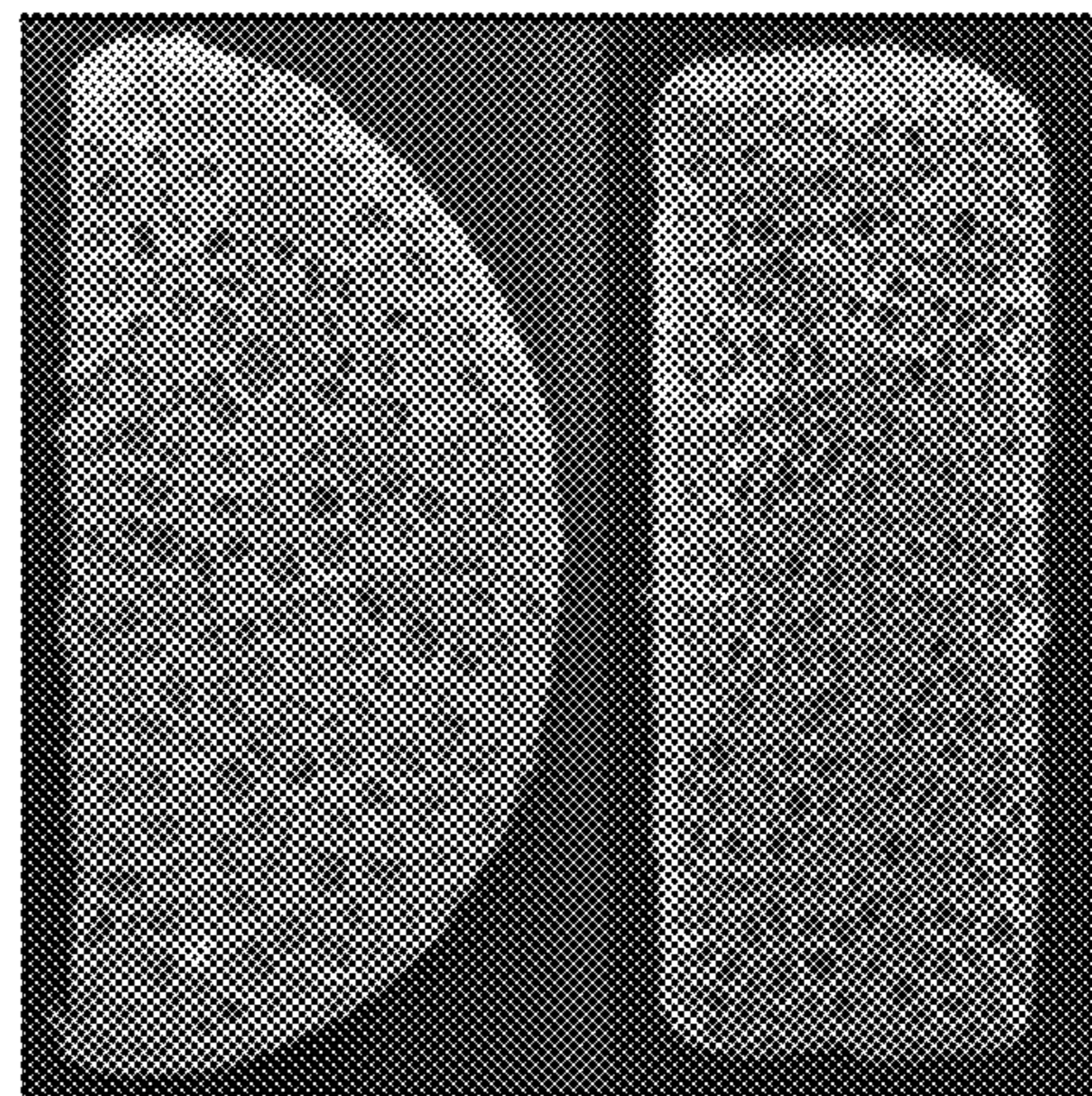
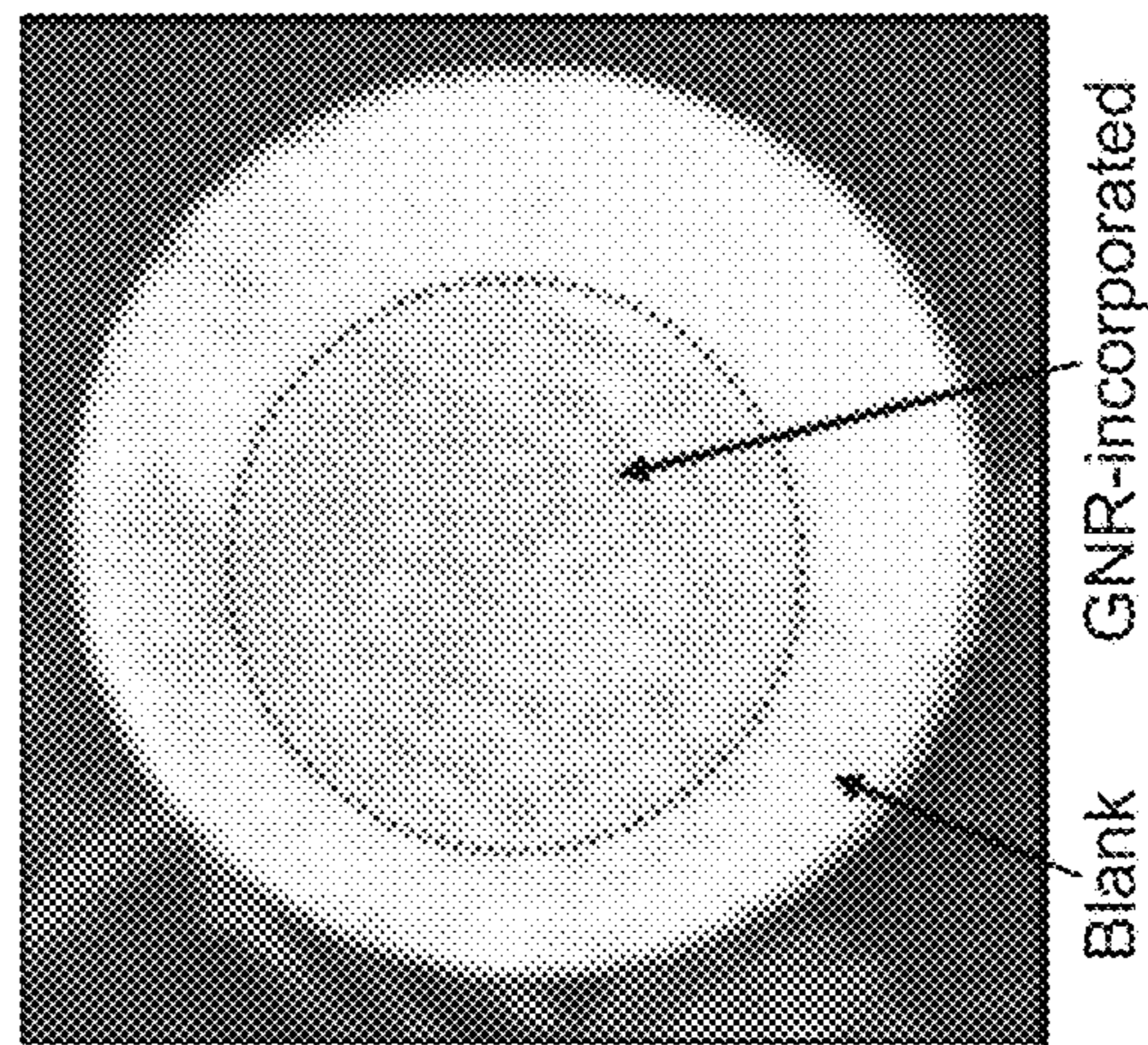


FIG. 10A



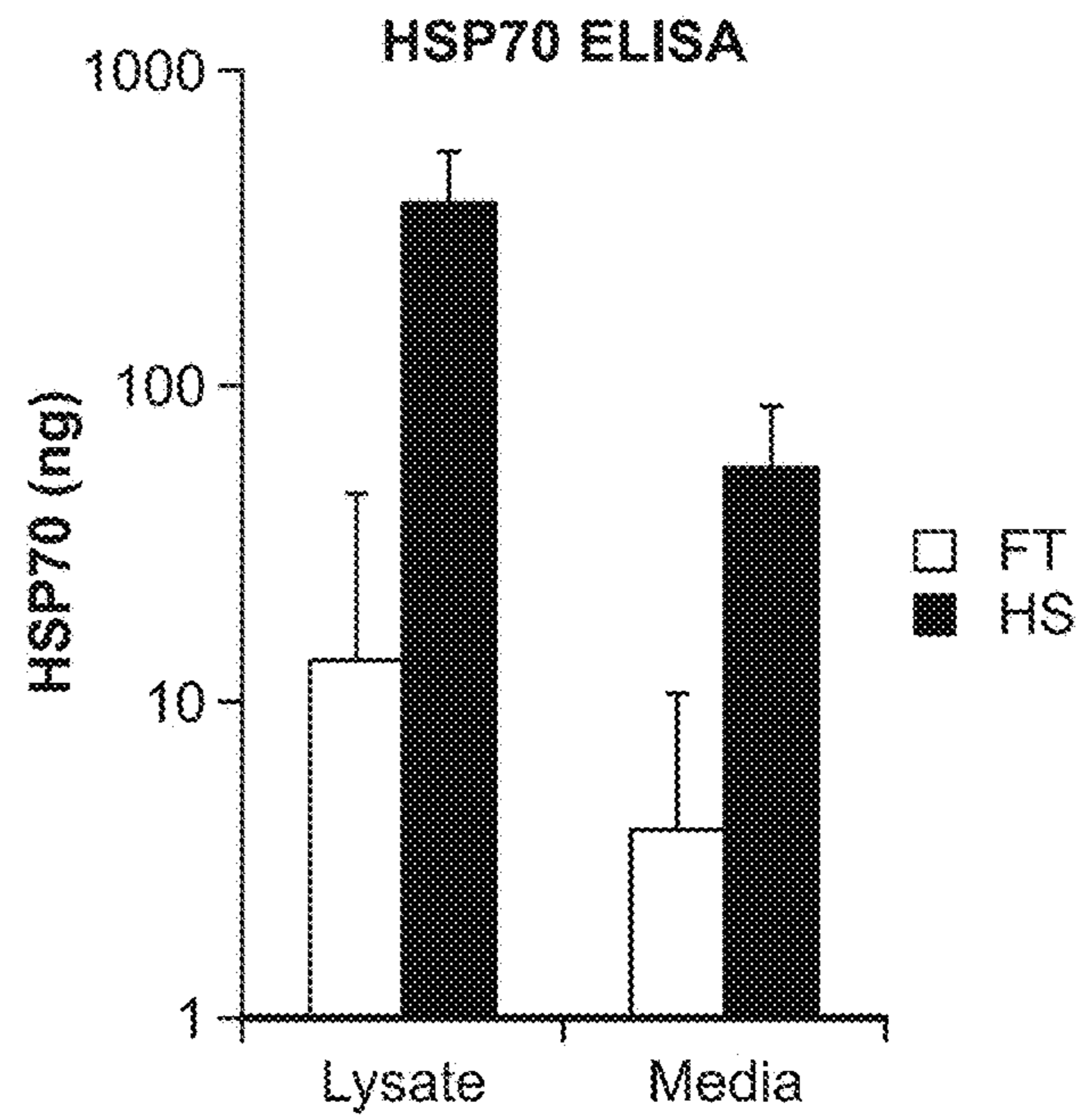


FIG. 11A

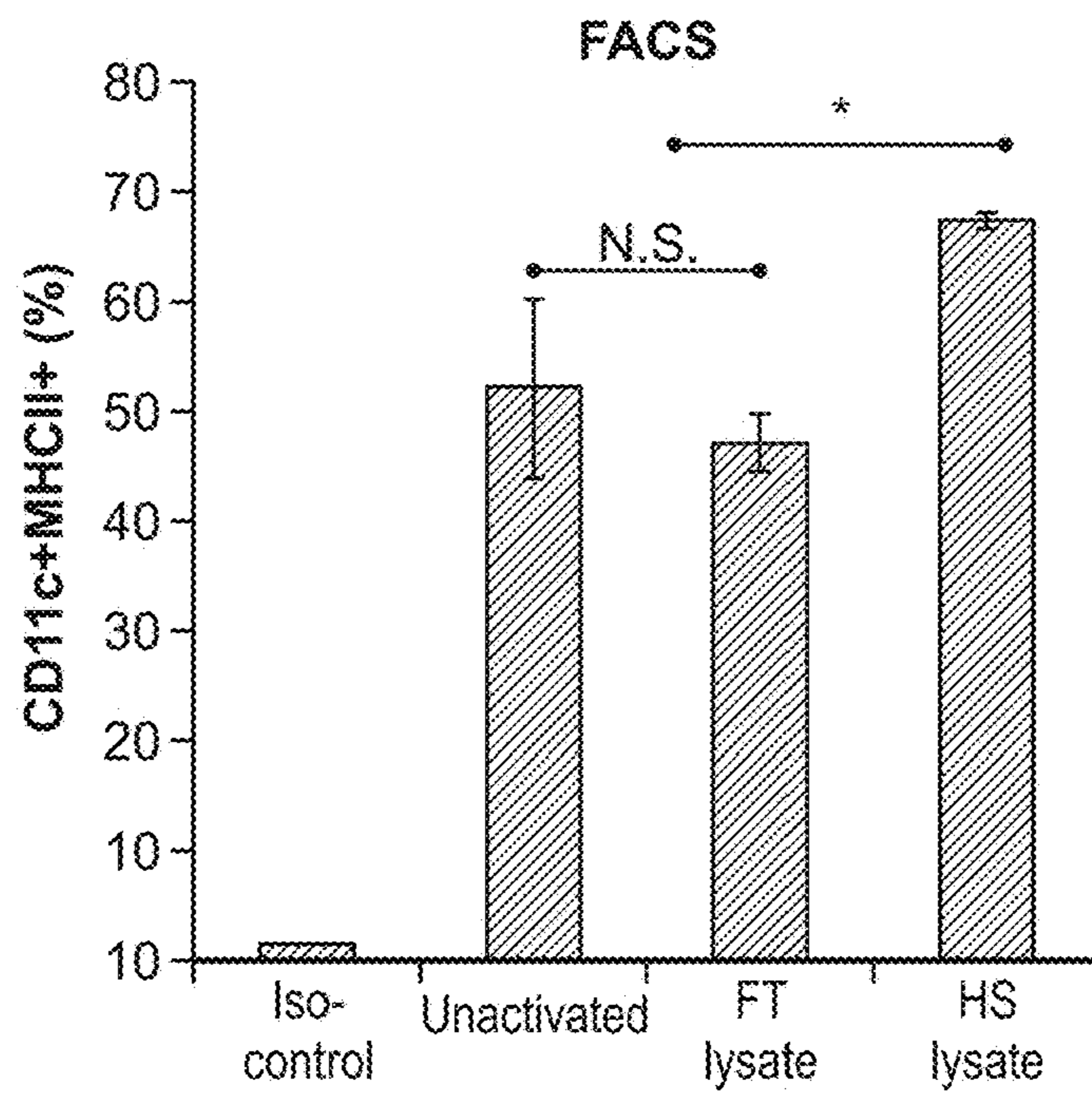


FIG. 11B

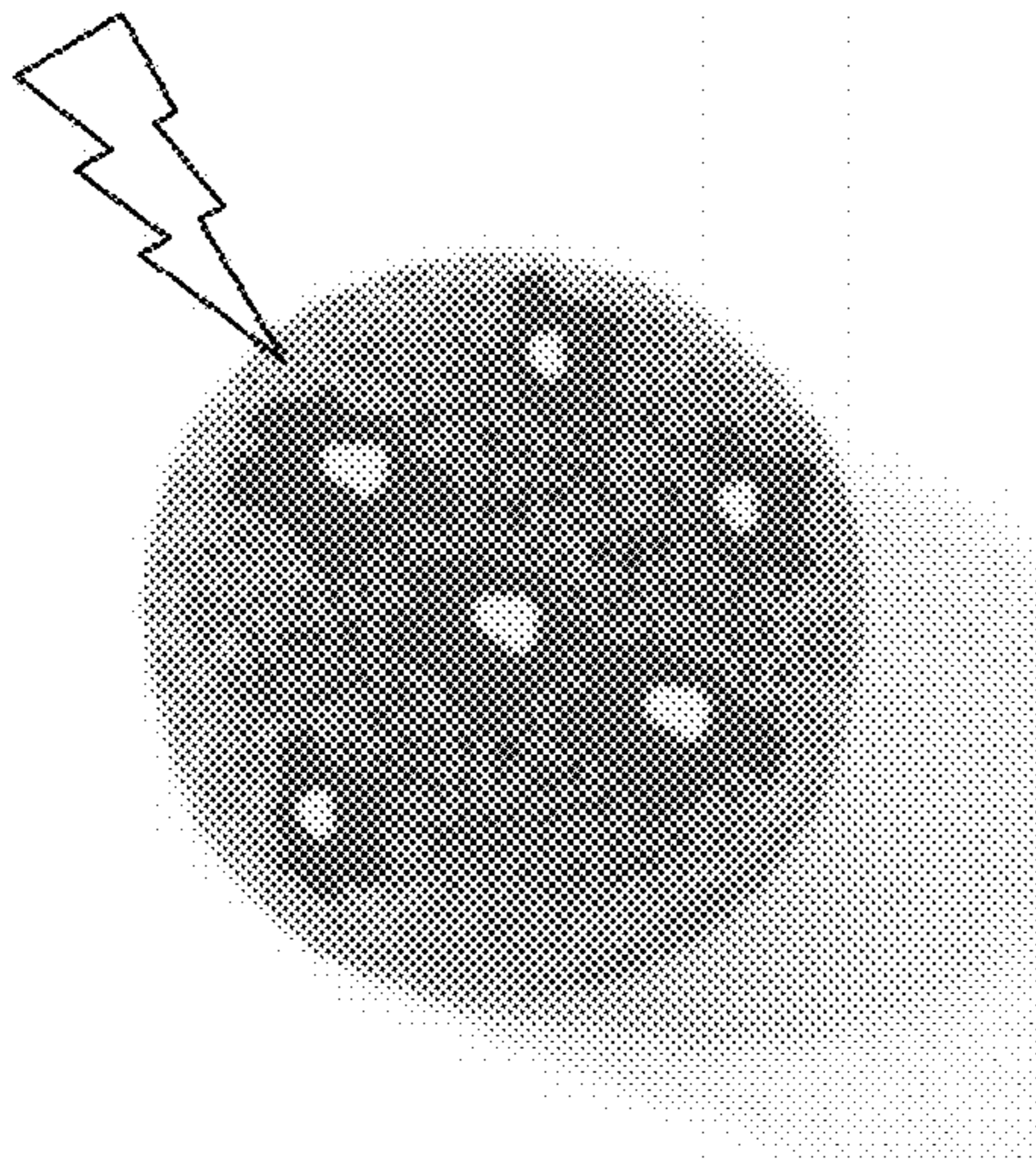


FIG. 12A

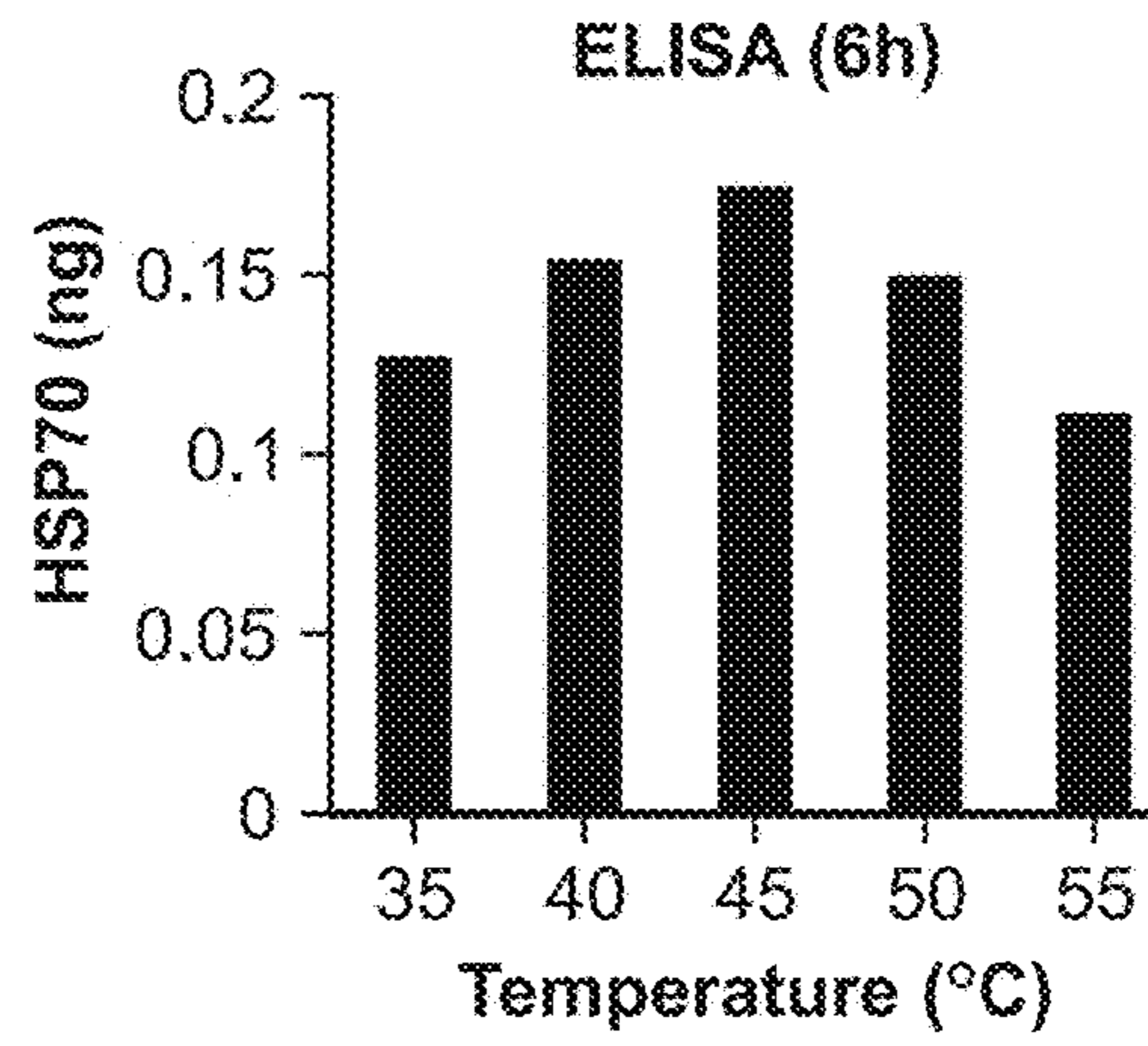


FIG. 12B

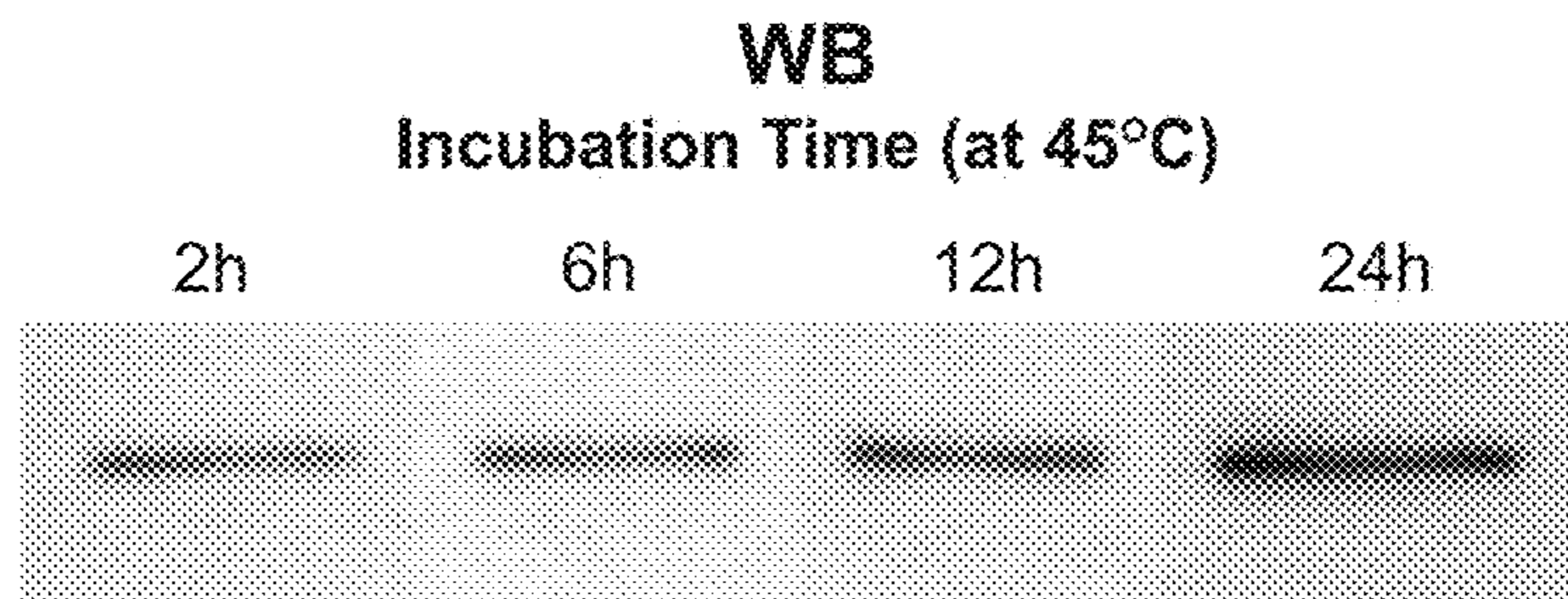


FIG. 12C

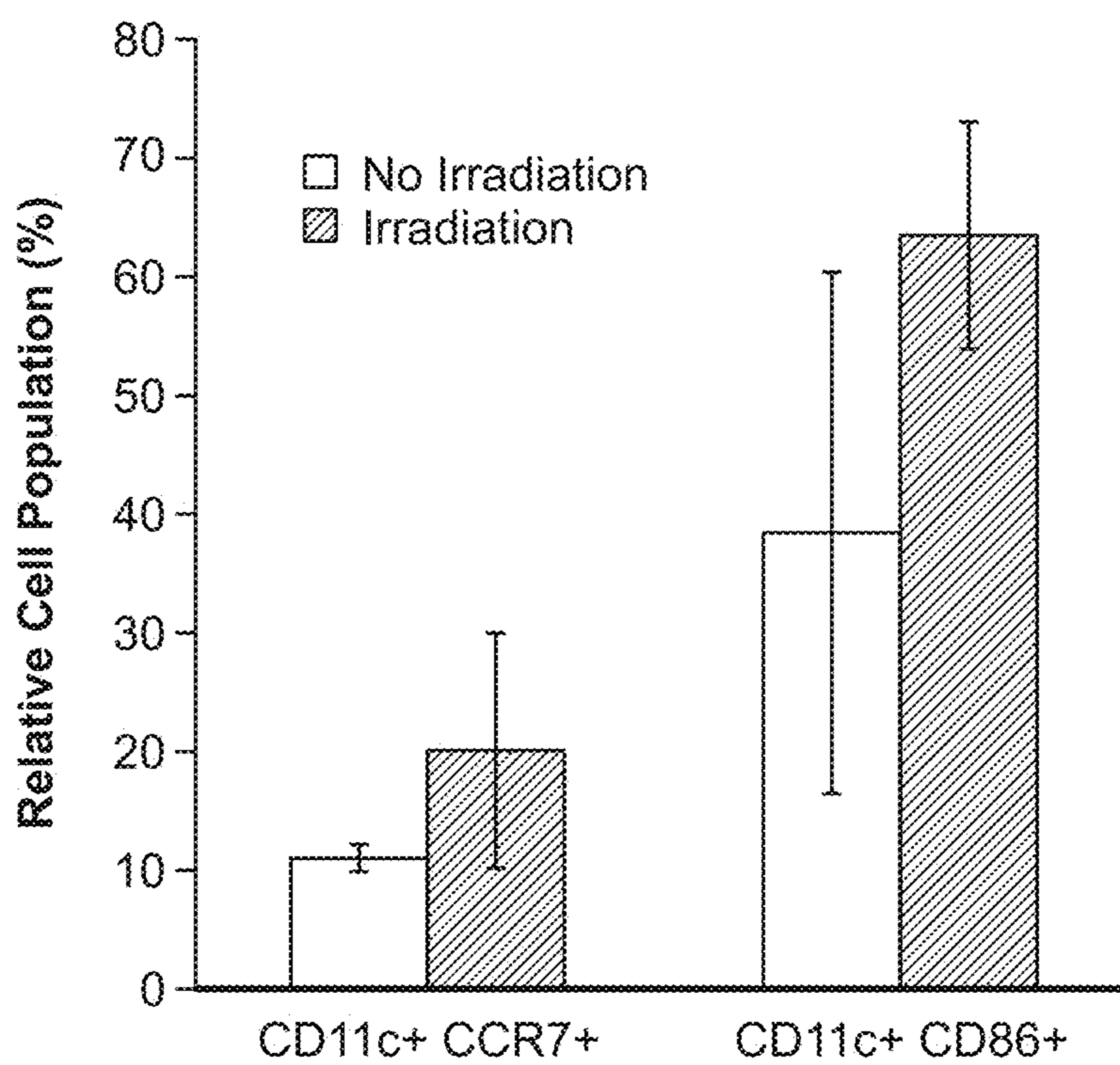


FIG. 13

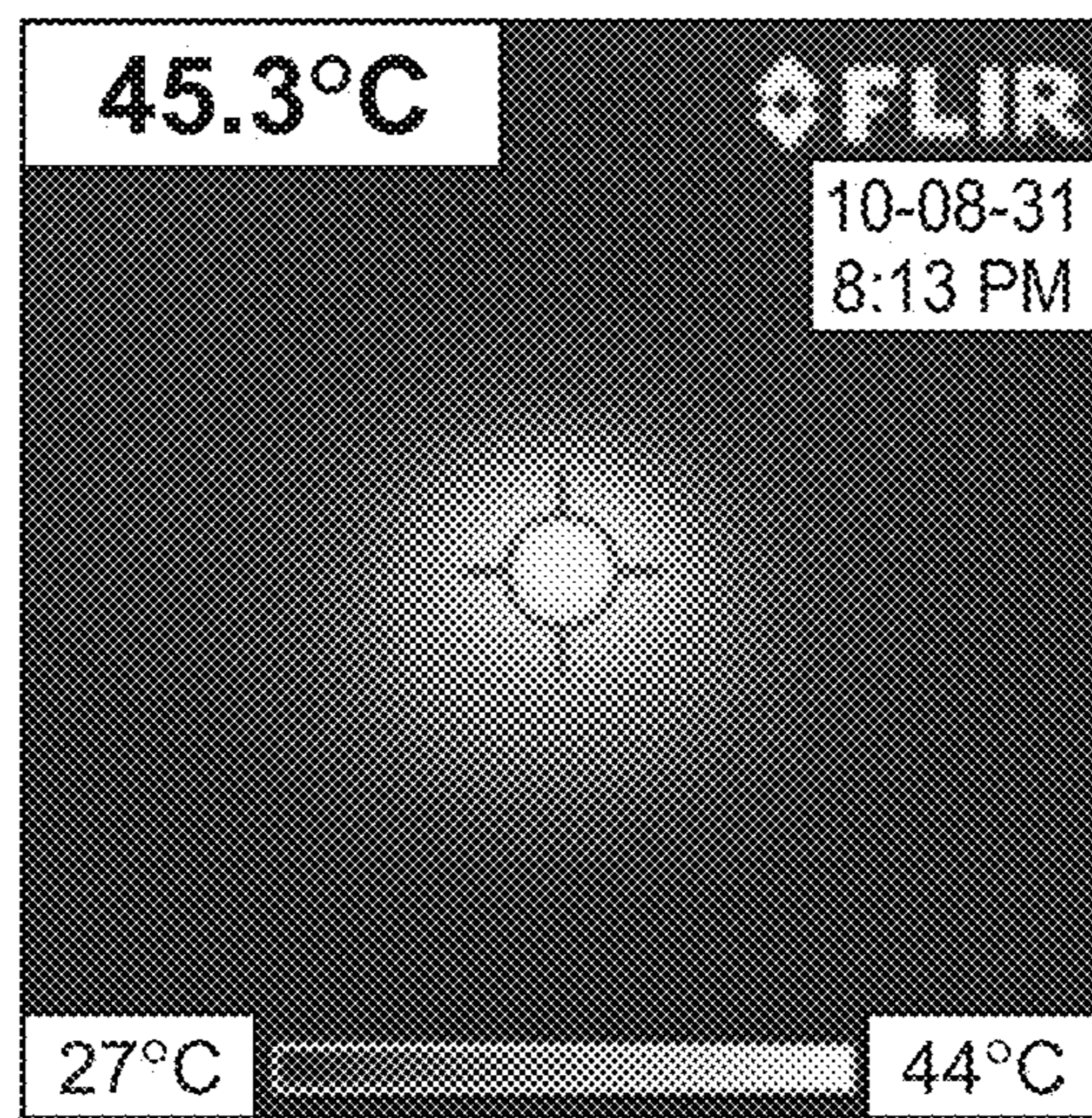


FIG. 14A

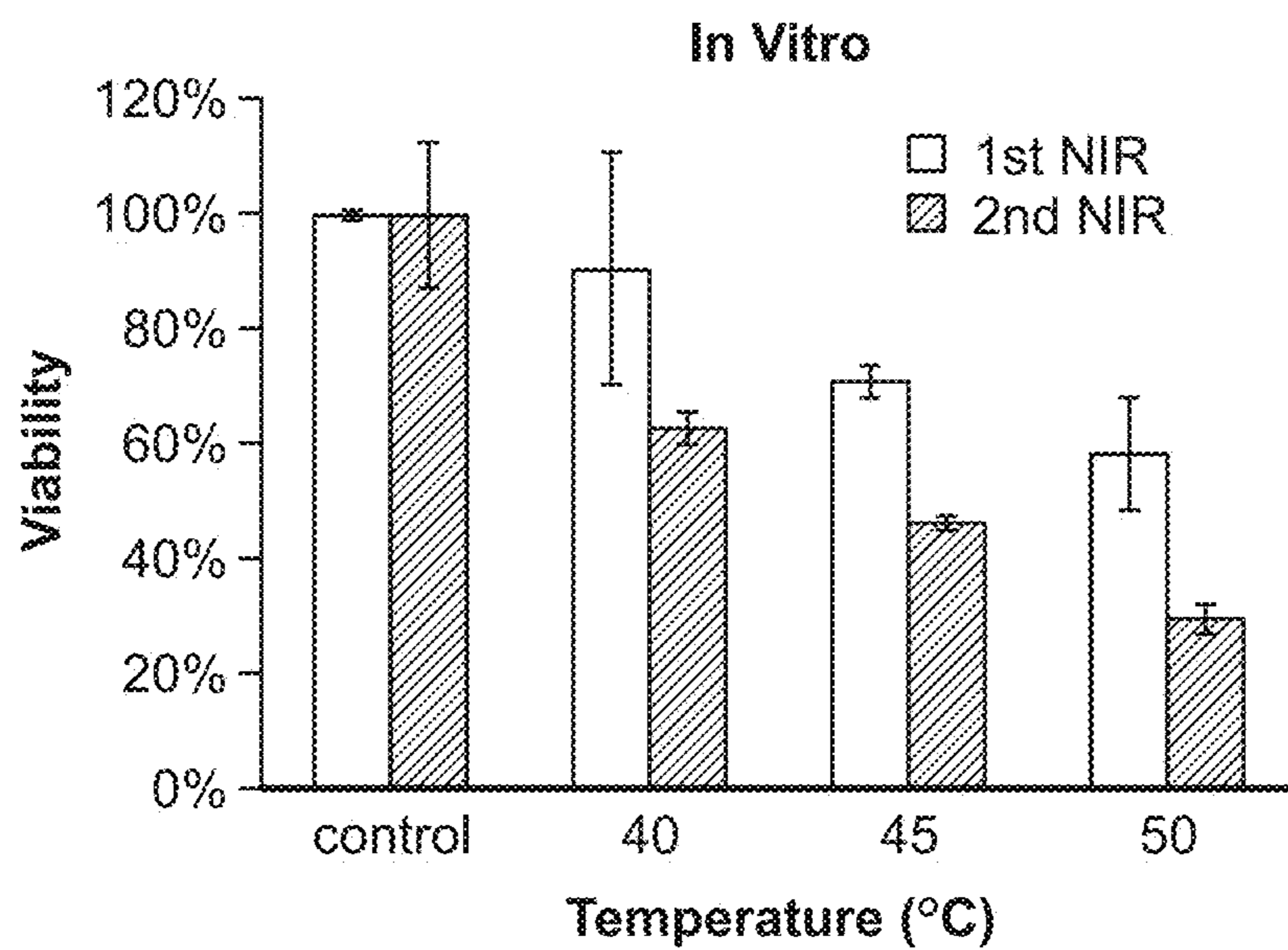


FIG. 14B

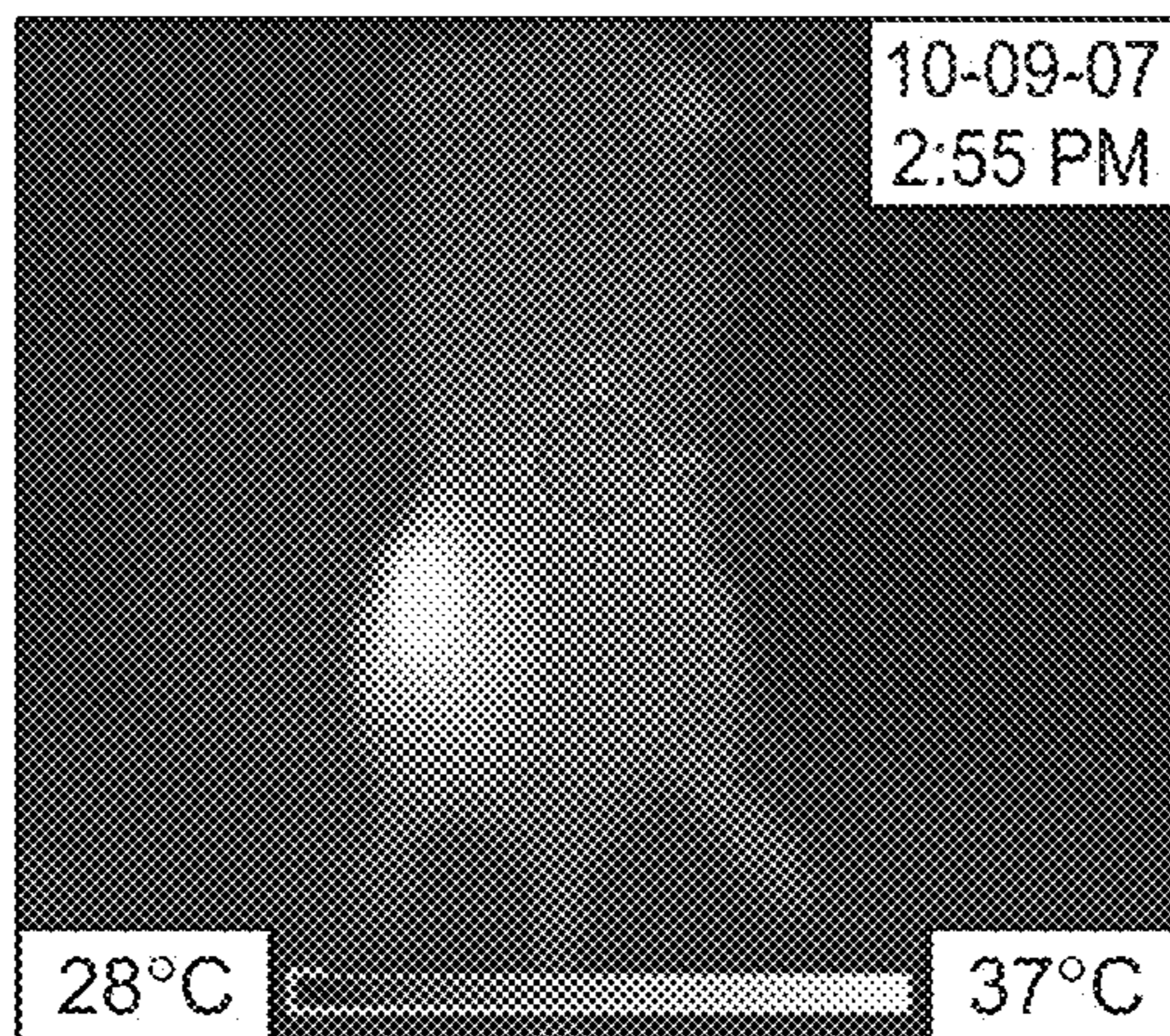


FIG. 15A

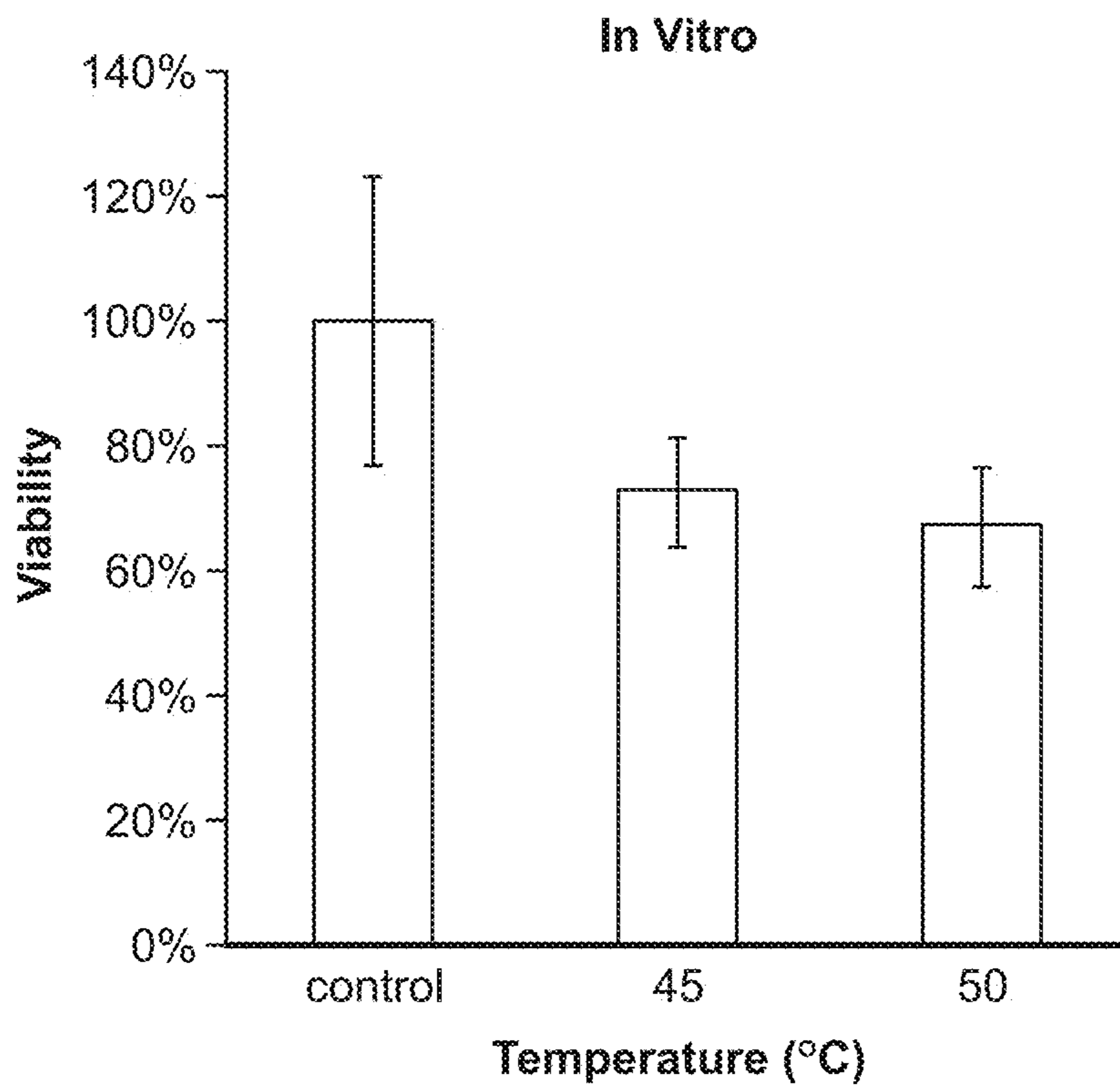


FIG. 15B

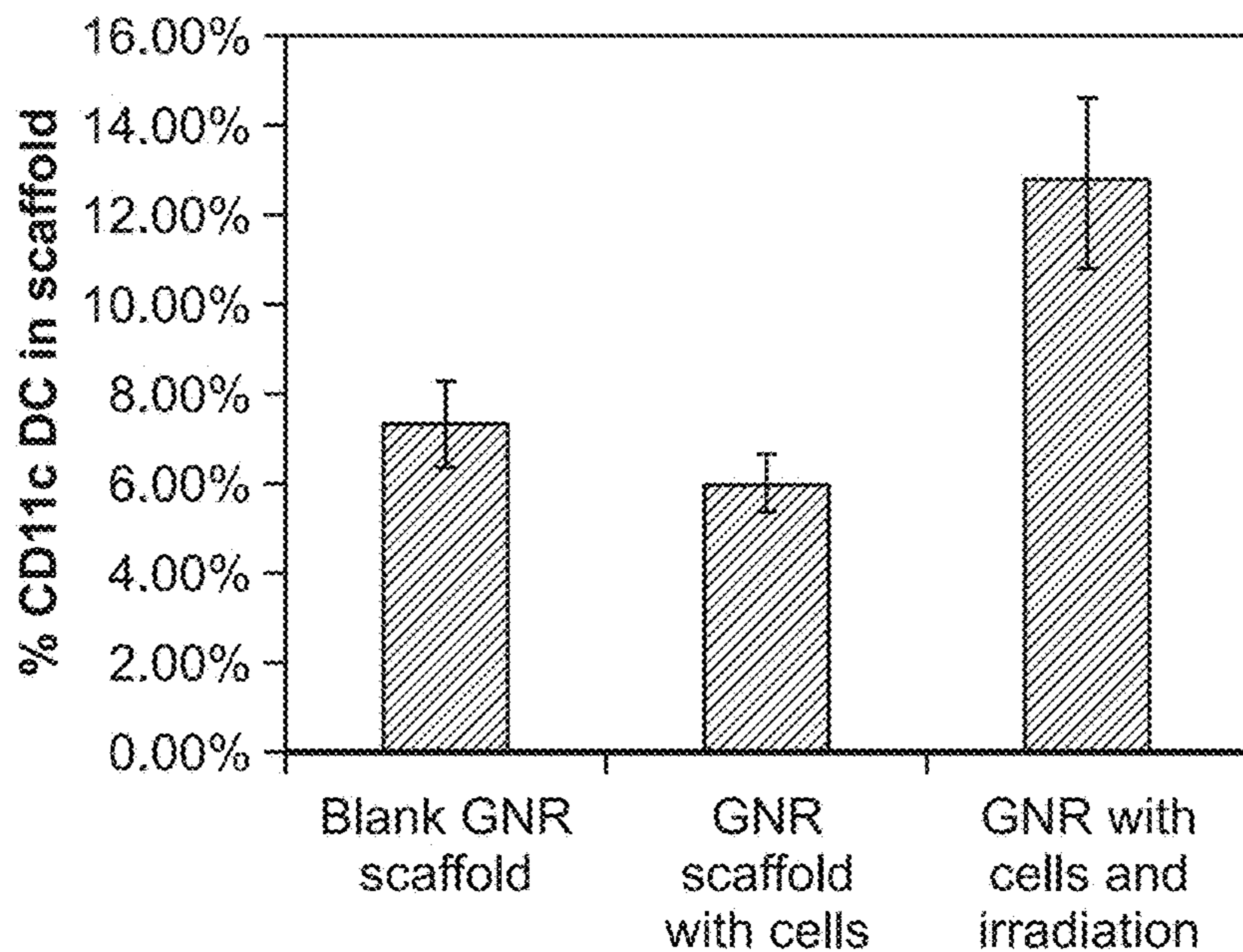


FIG. 16A

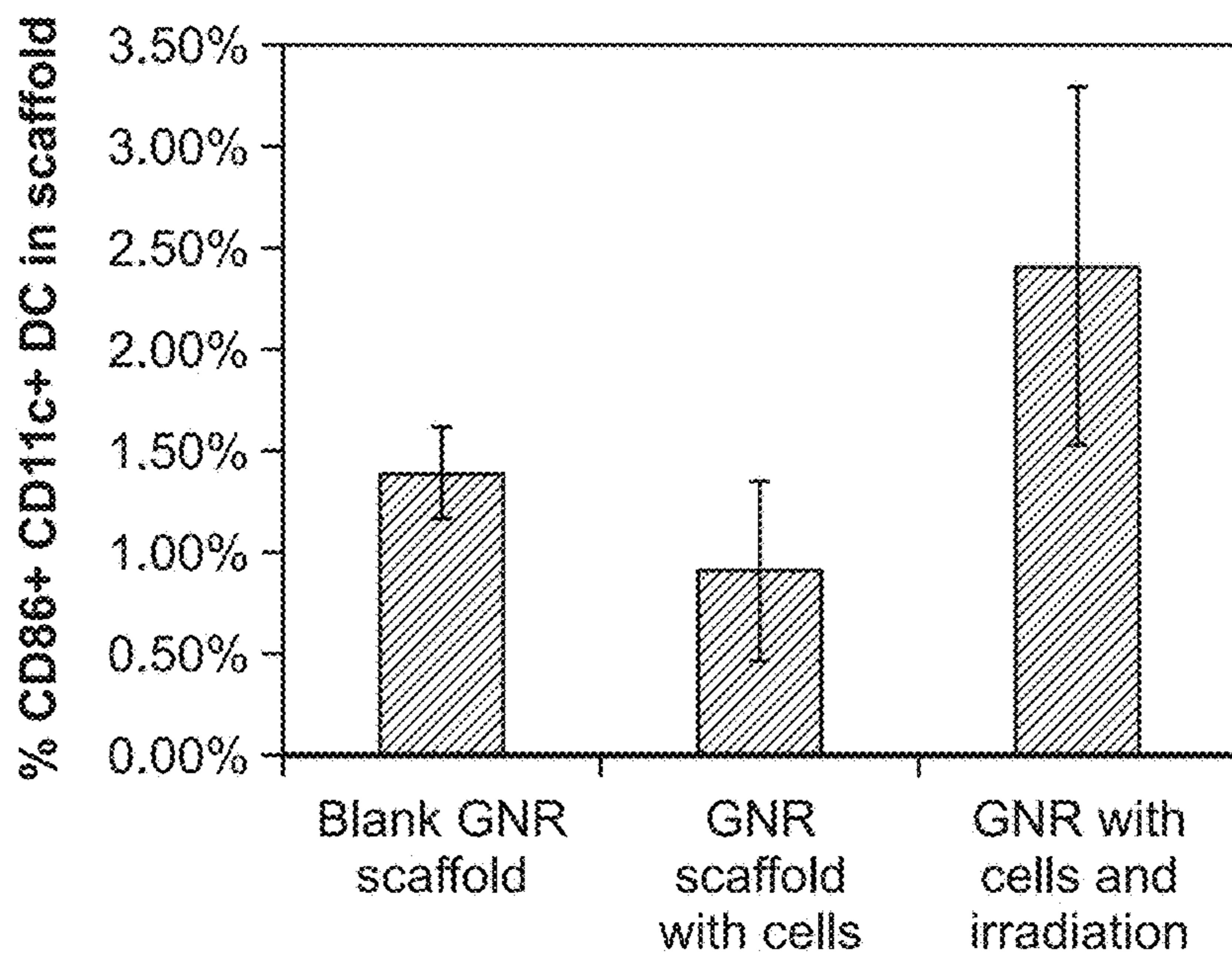


FIG. 16B

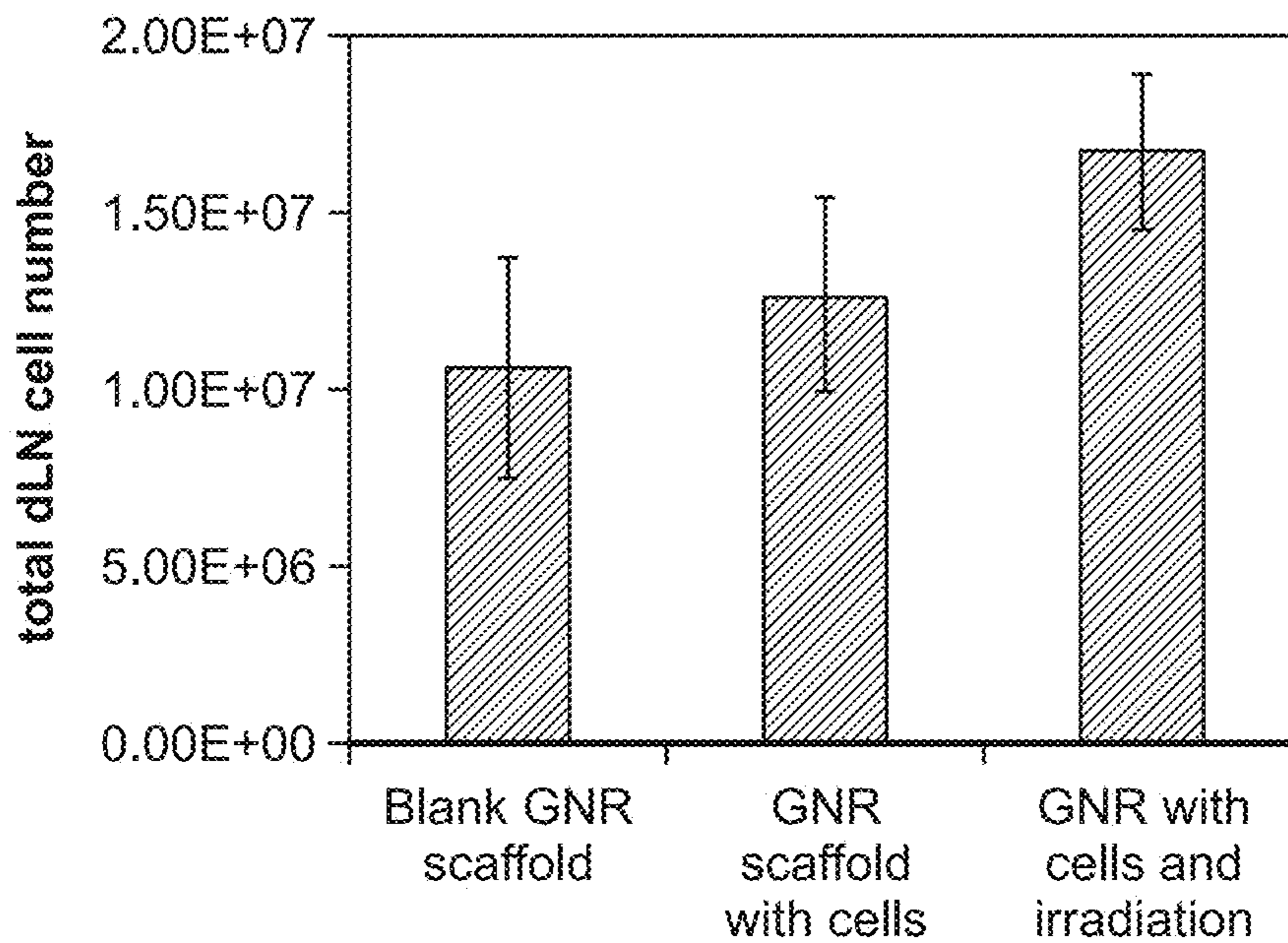


FIG. 17A

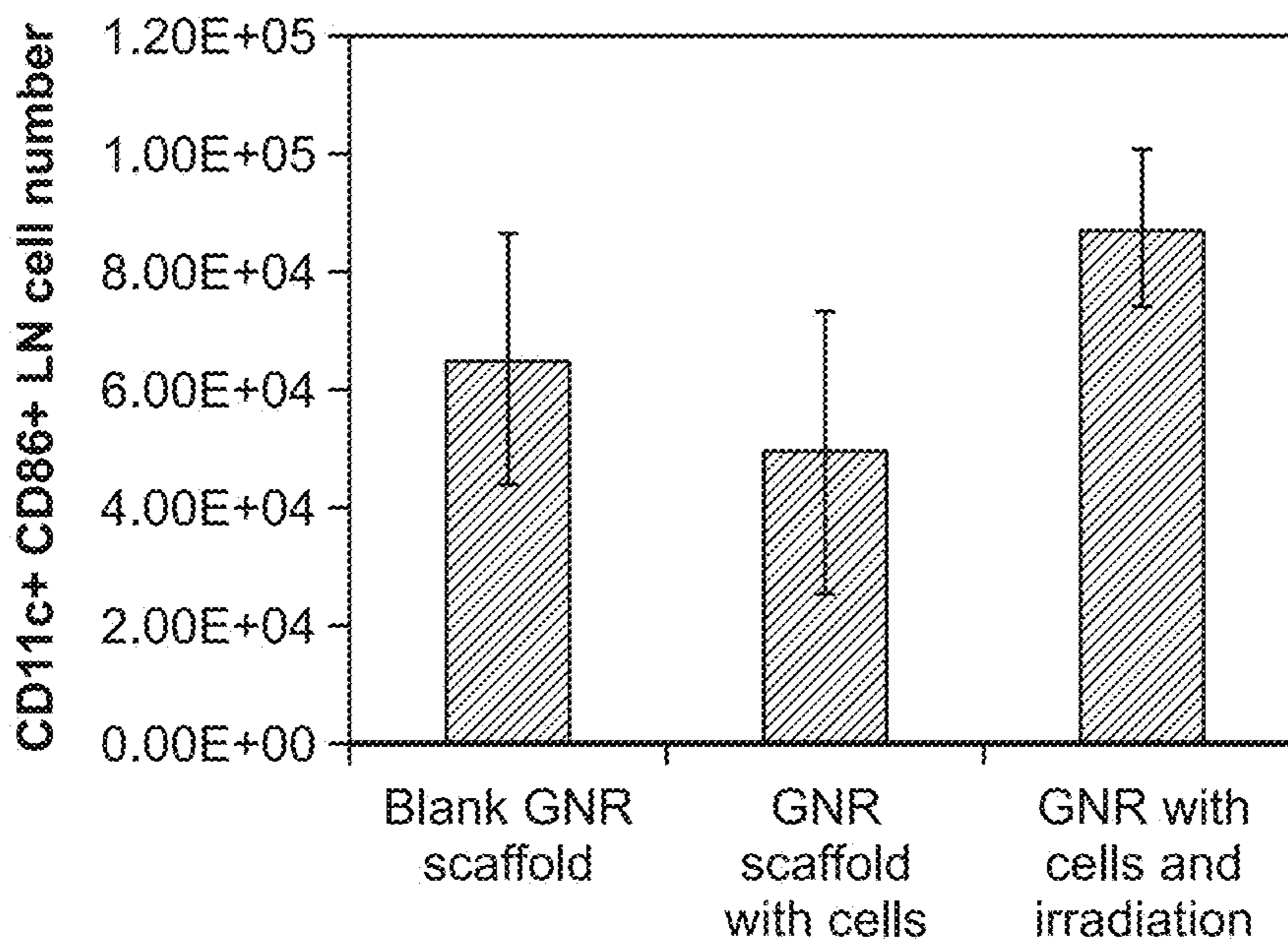


FIG. 17B

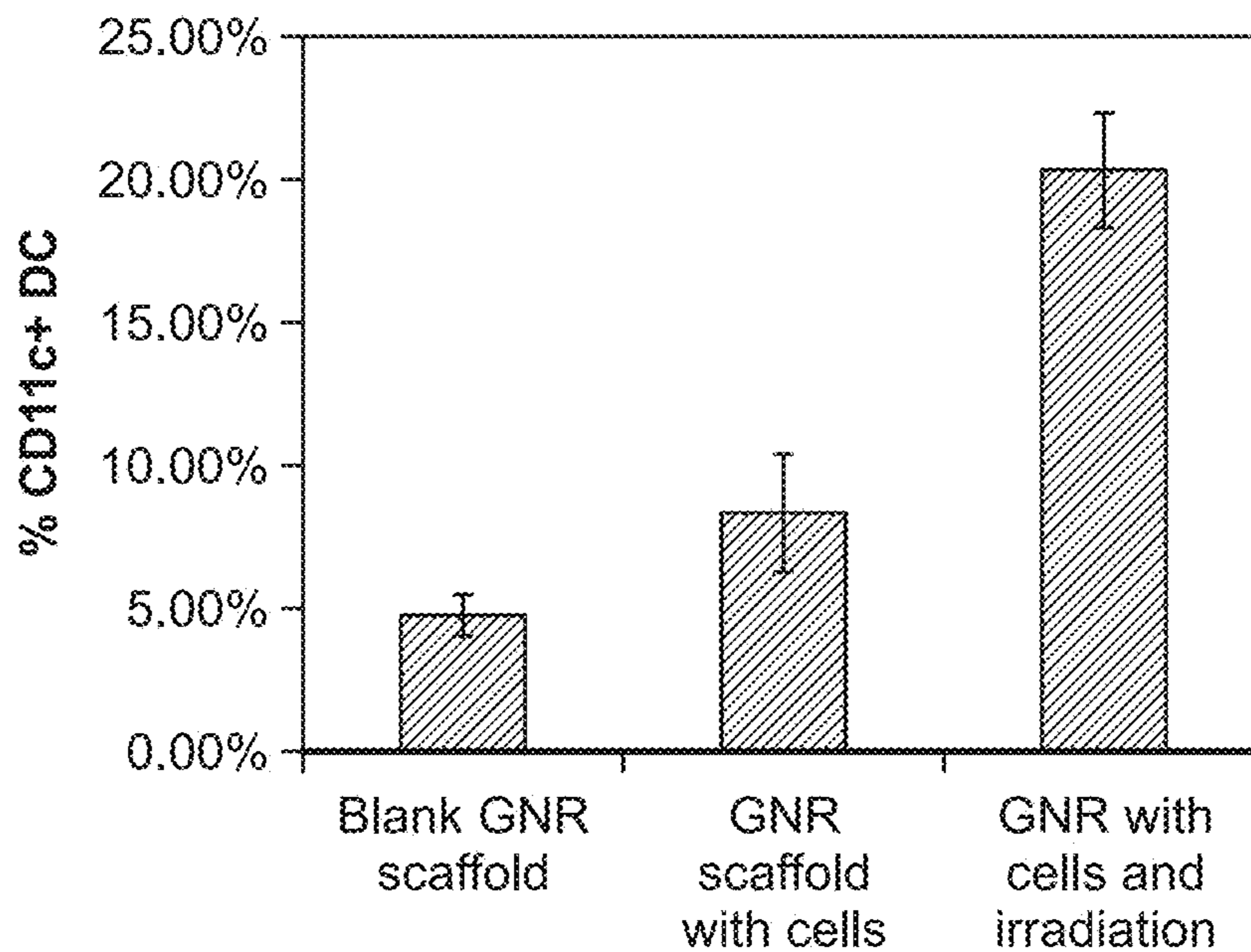


FIG. 18A

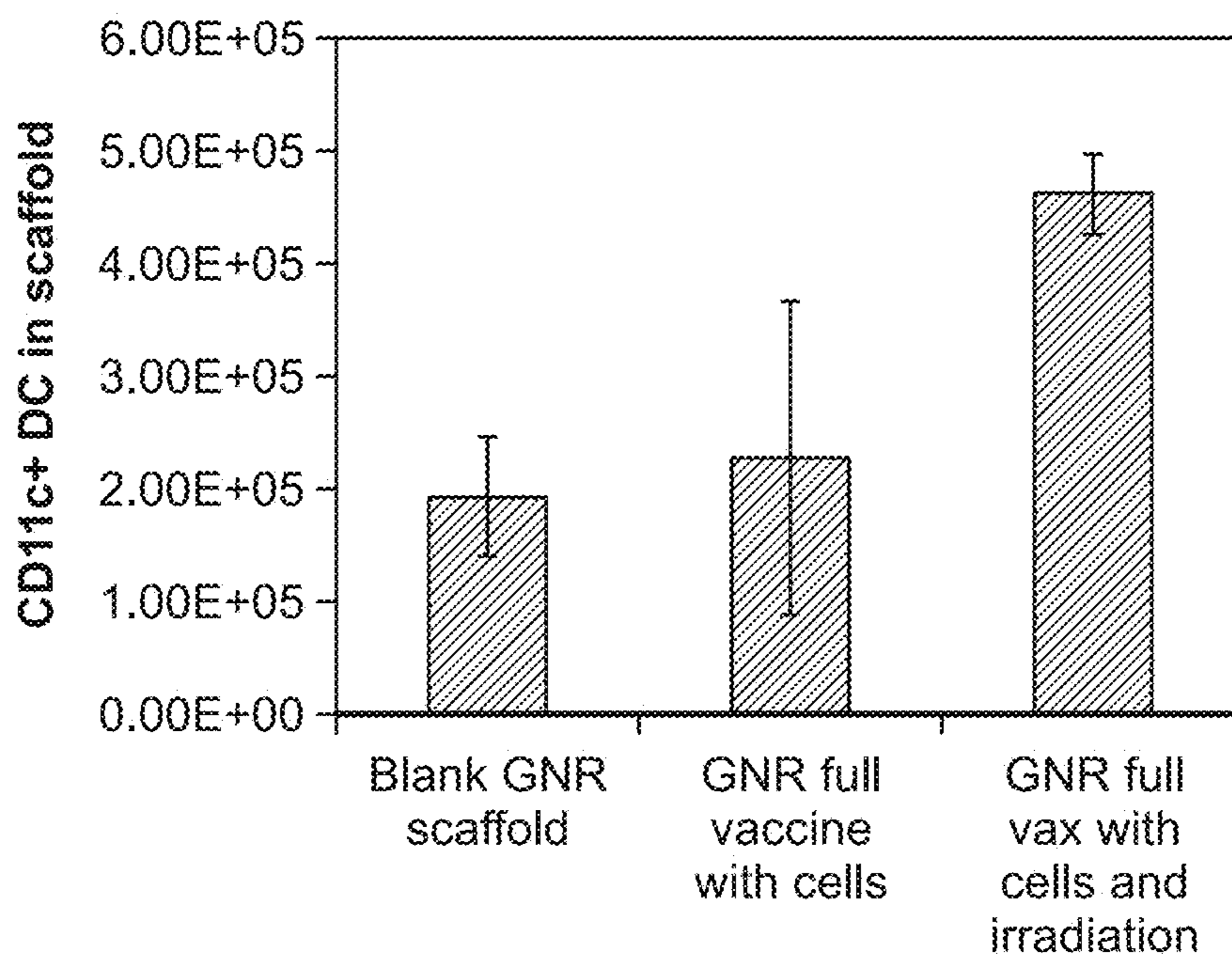


FIG. 18B

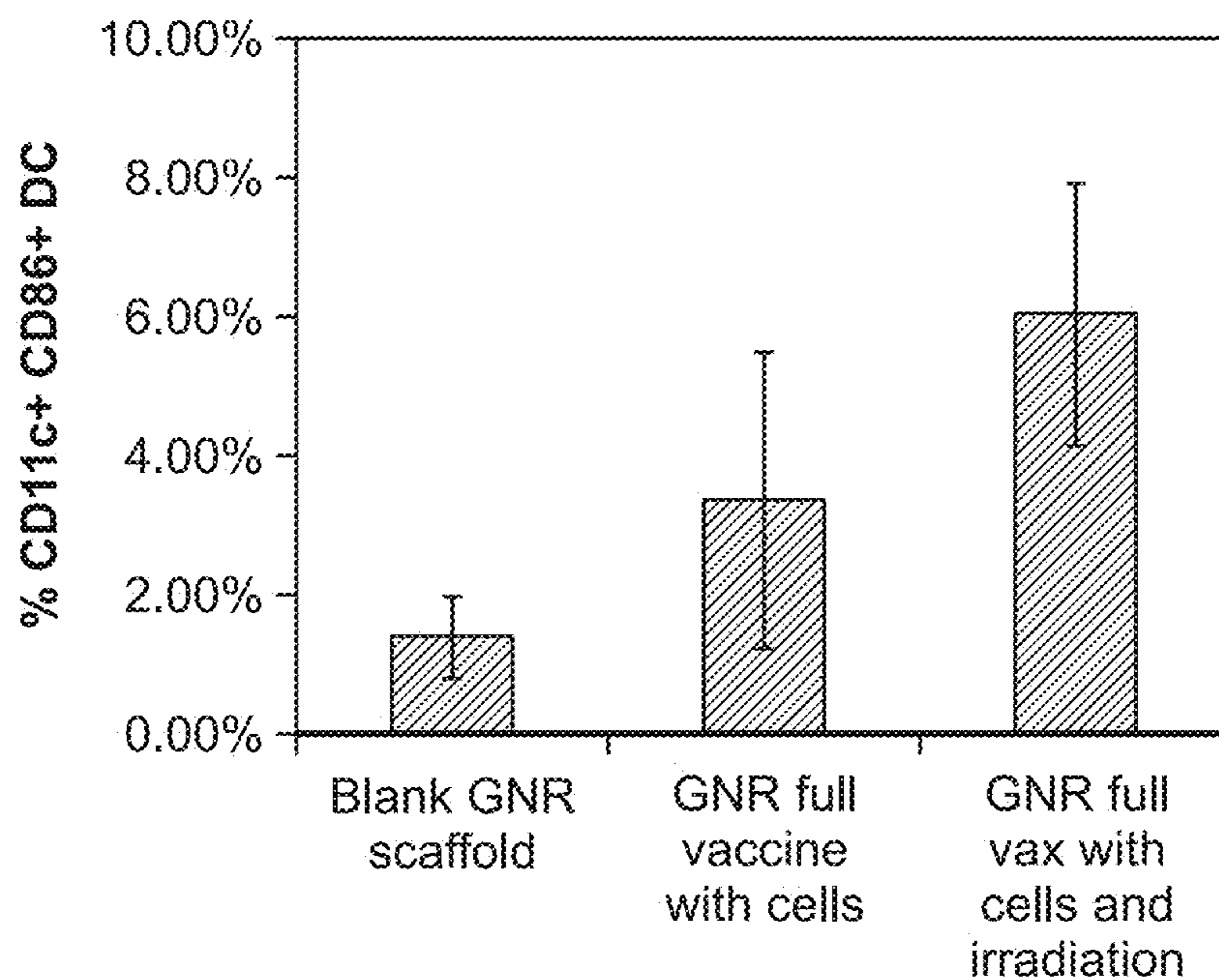


FIG. 18C

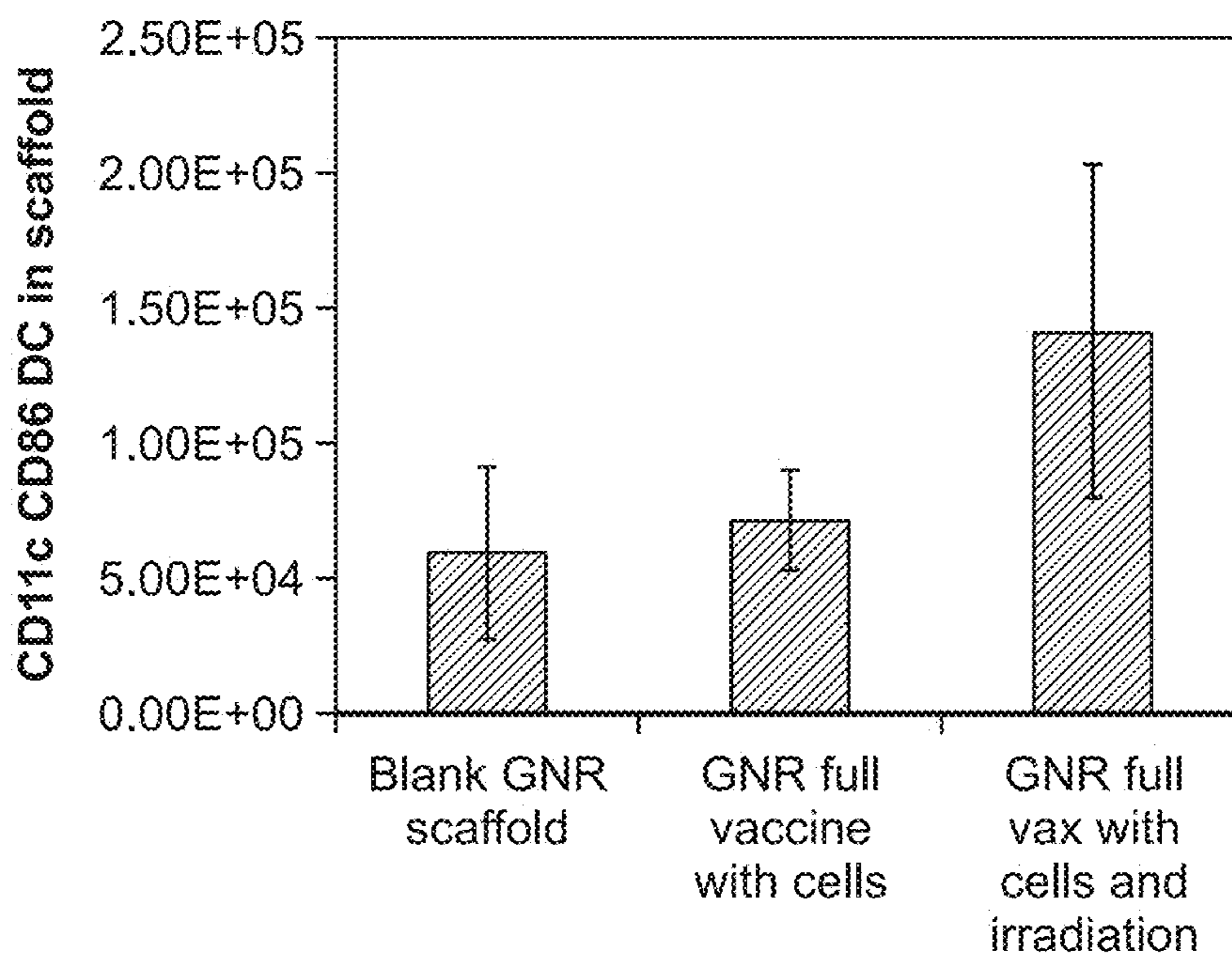


FIG. 18D

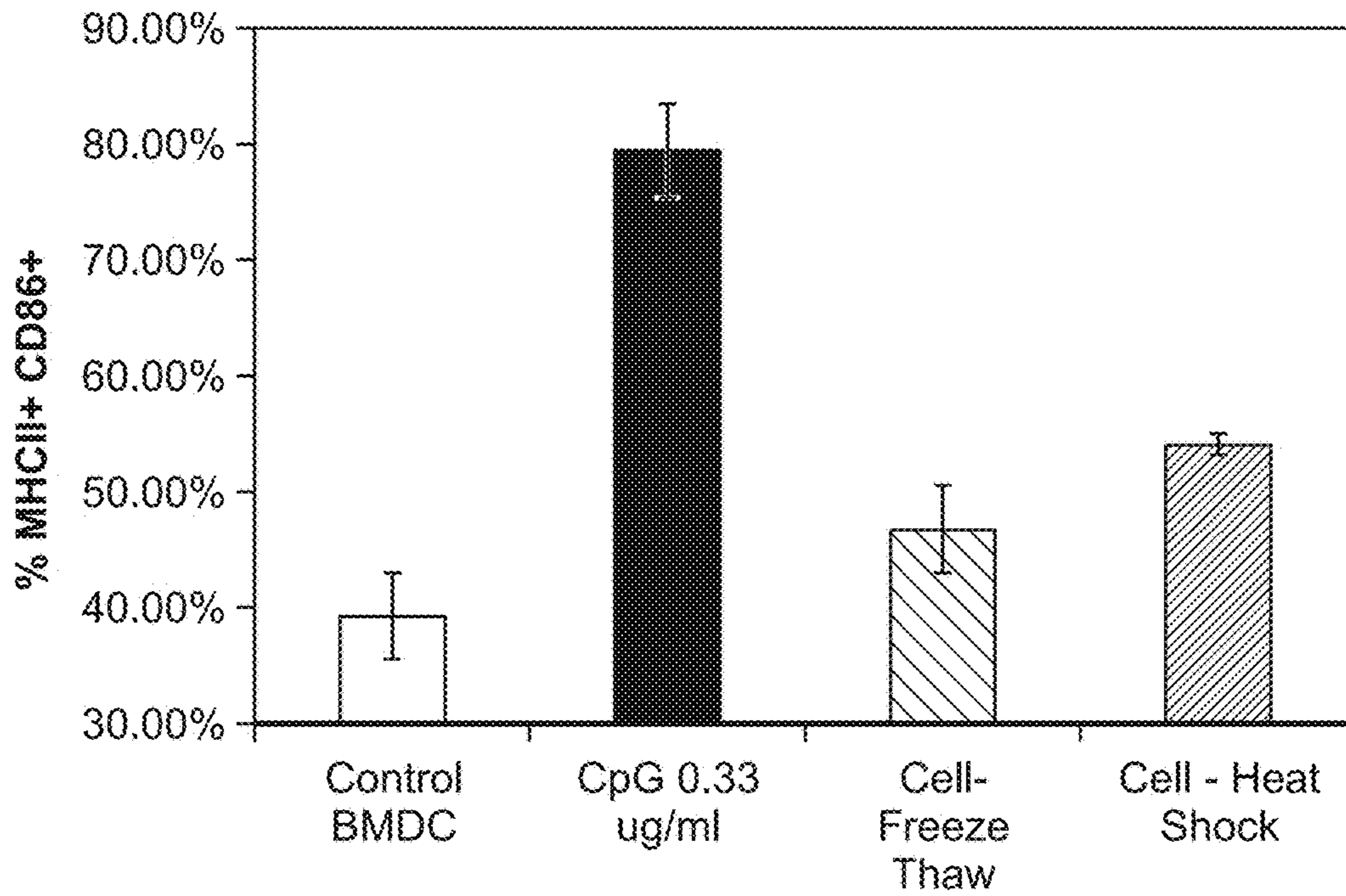


FIG. 19

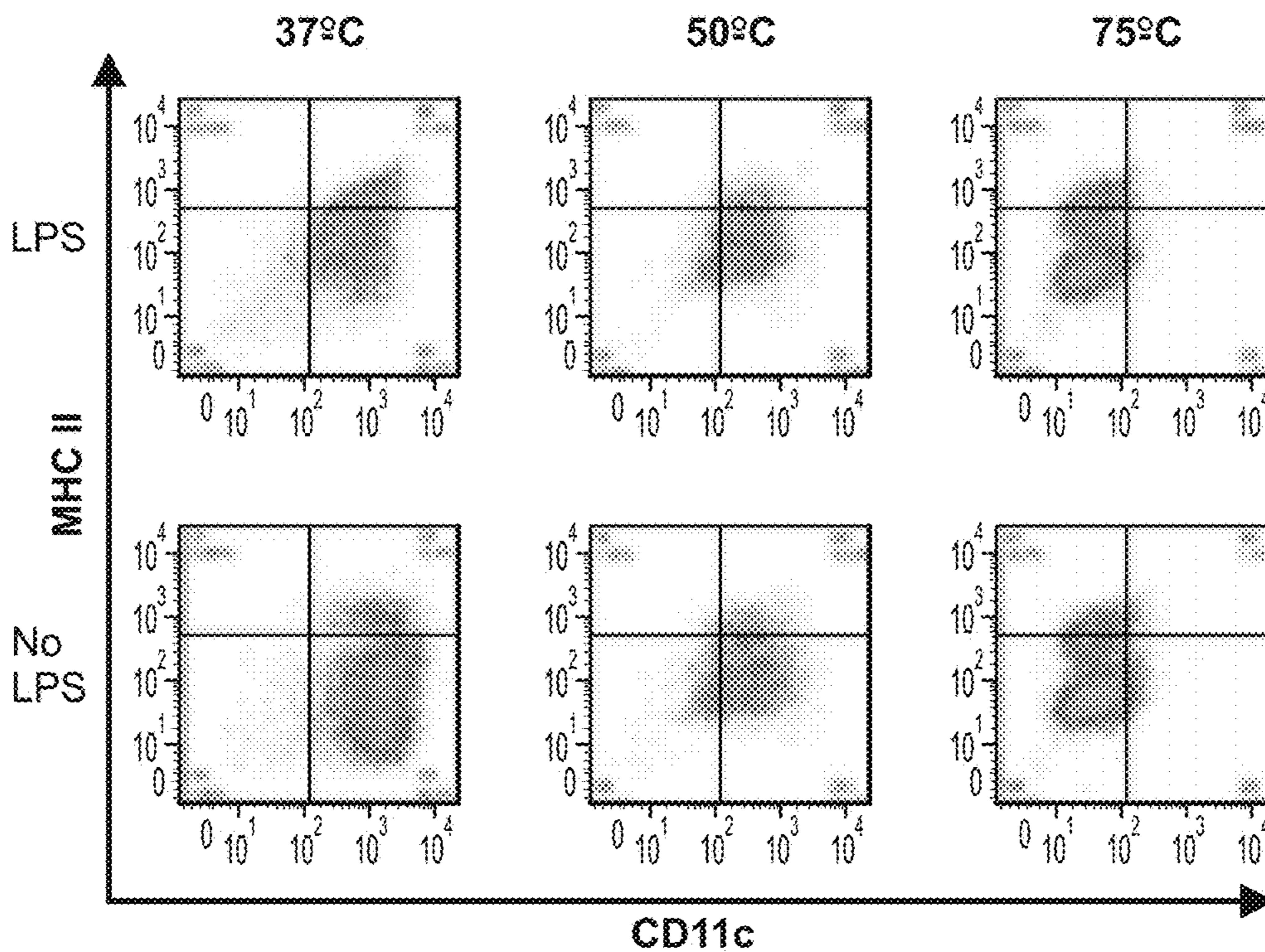


FIG. 20A

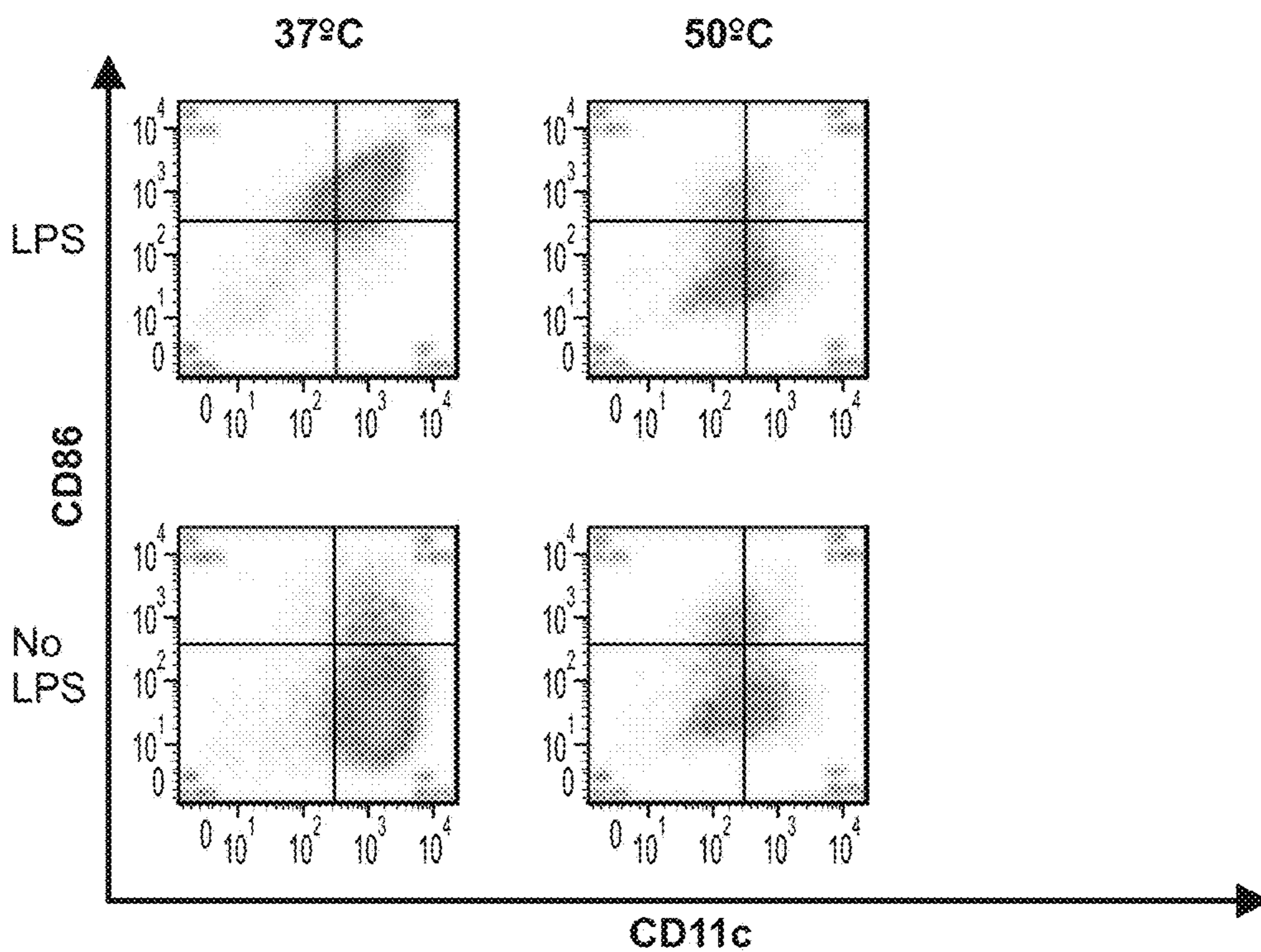


FIG. 20B

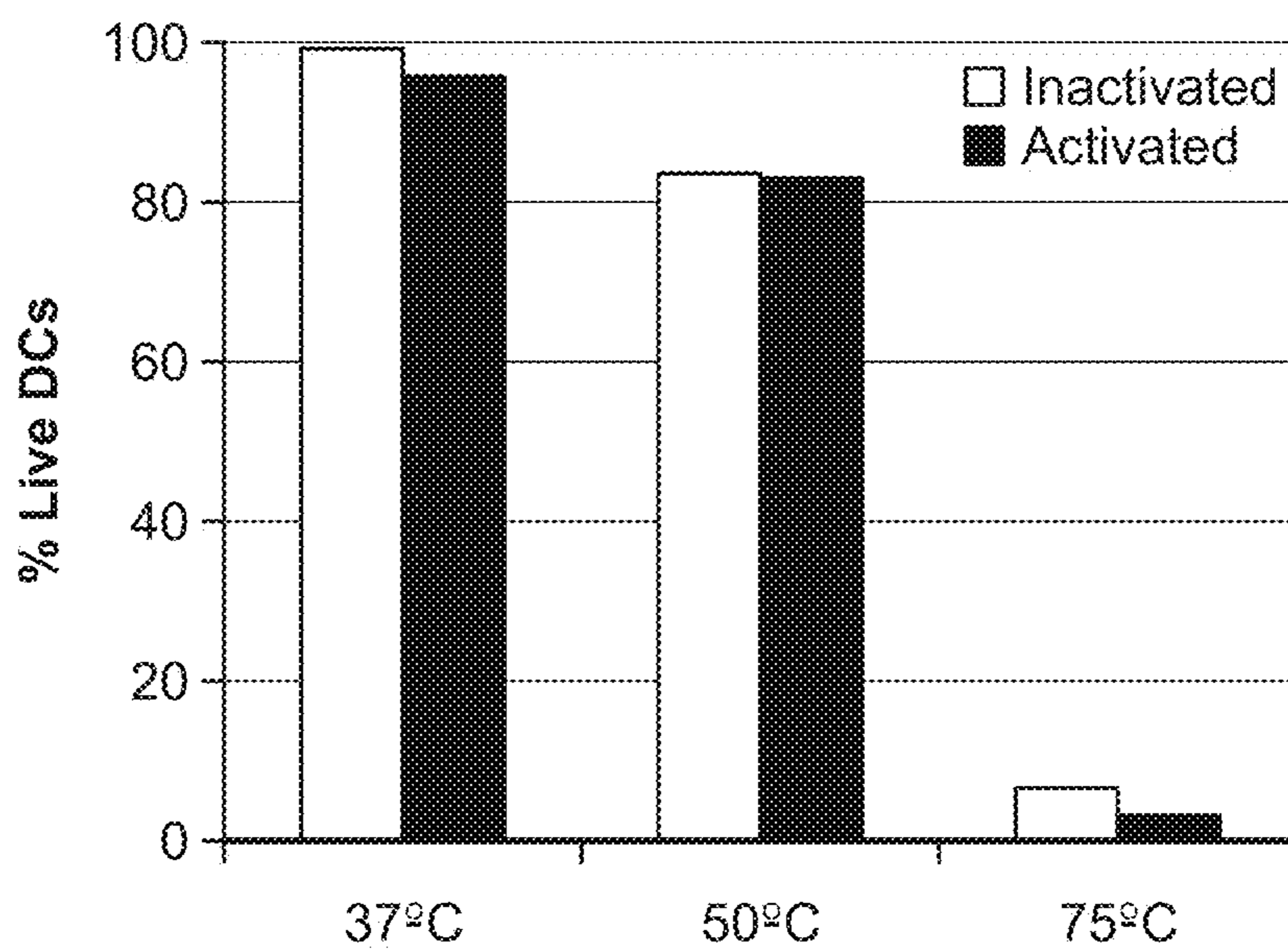


FIG. 20C

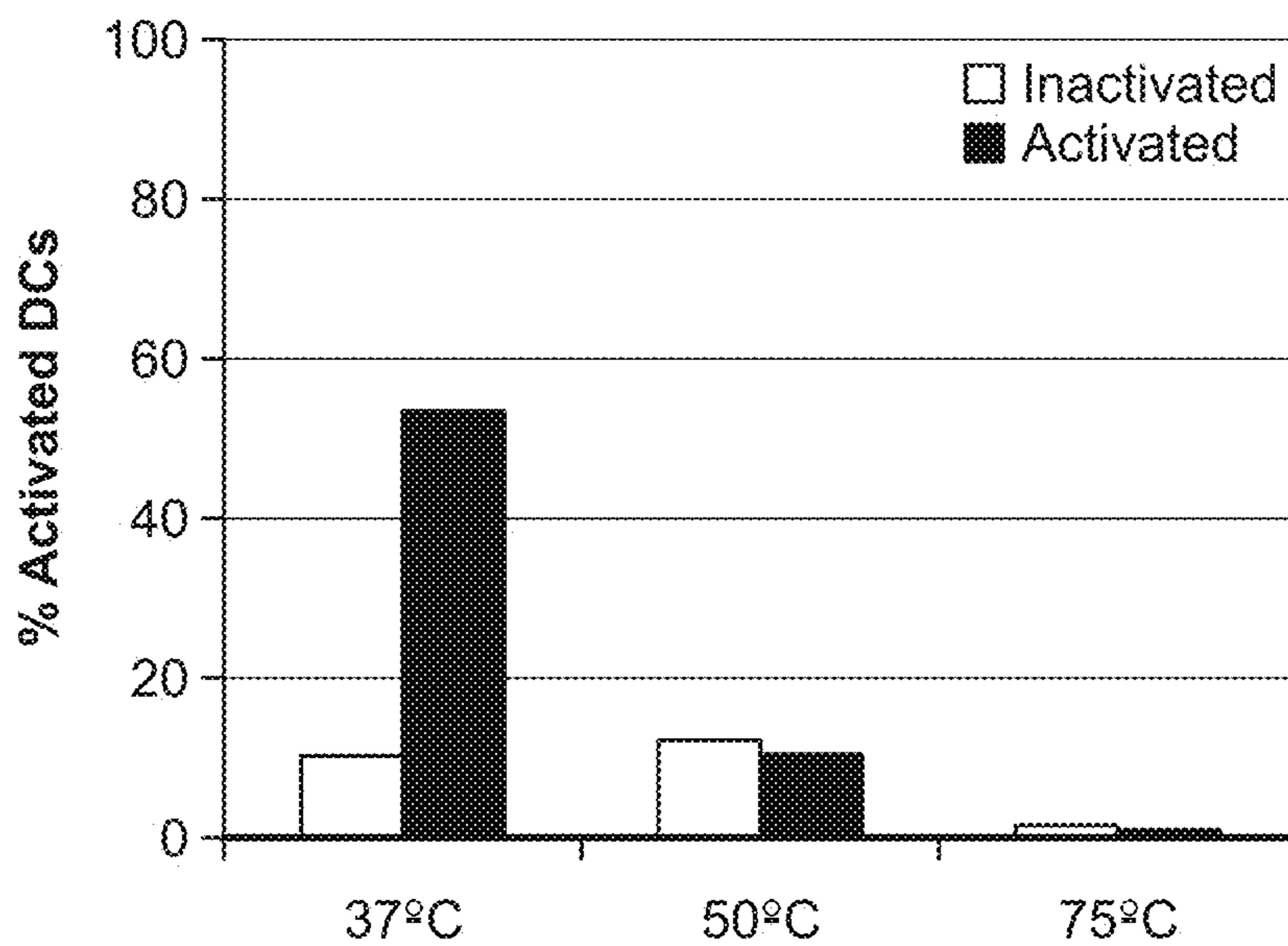


FIG. 20D

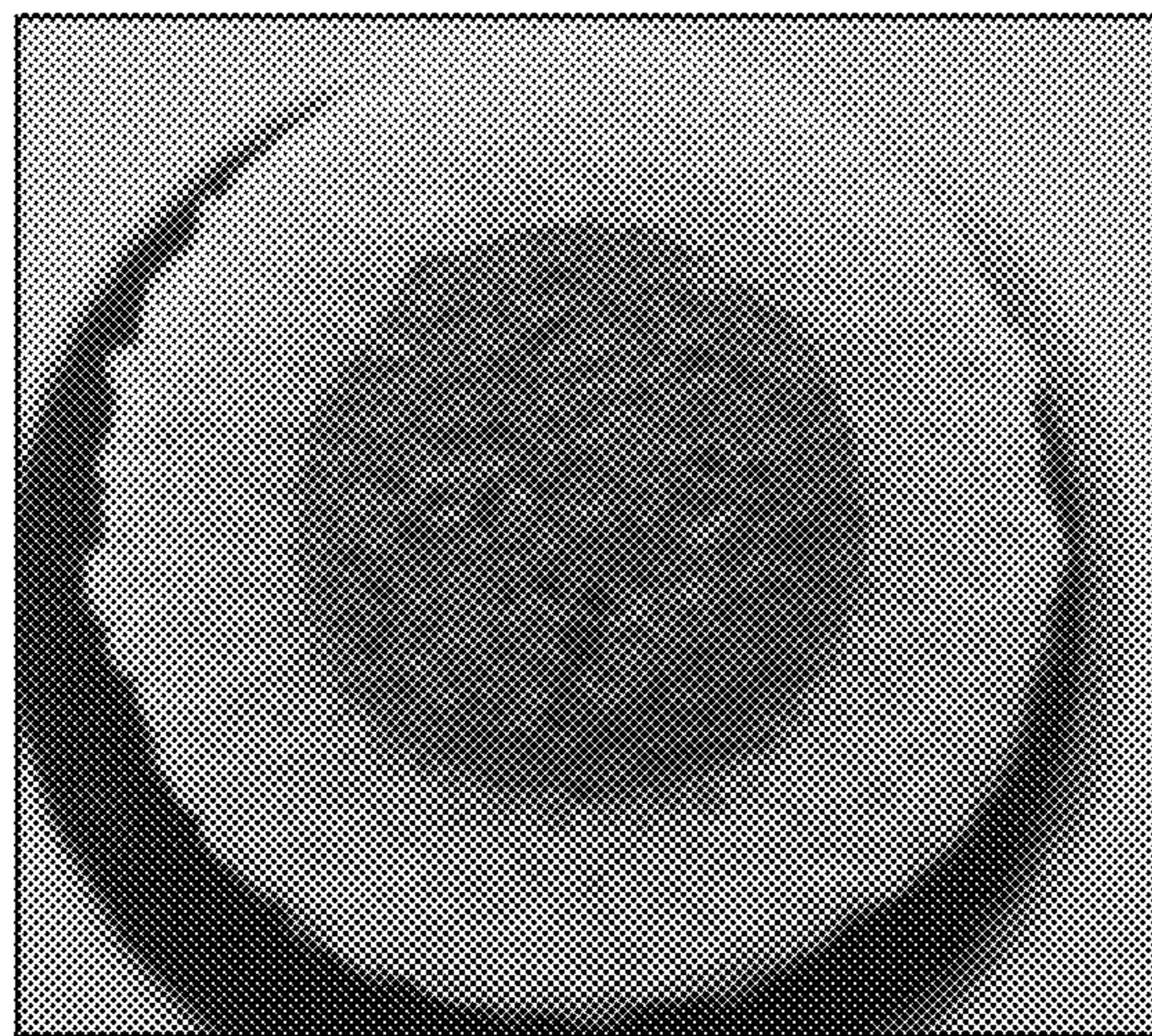


FIG. 21A

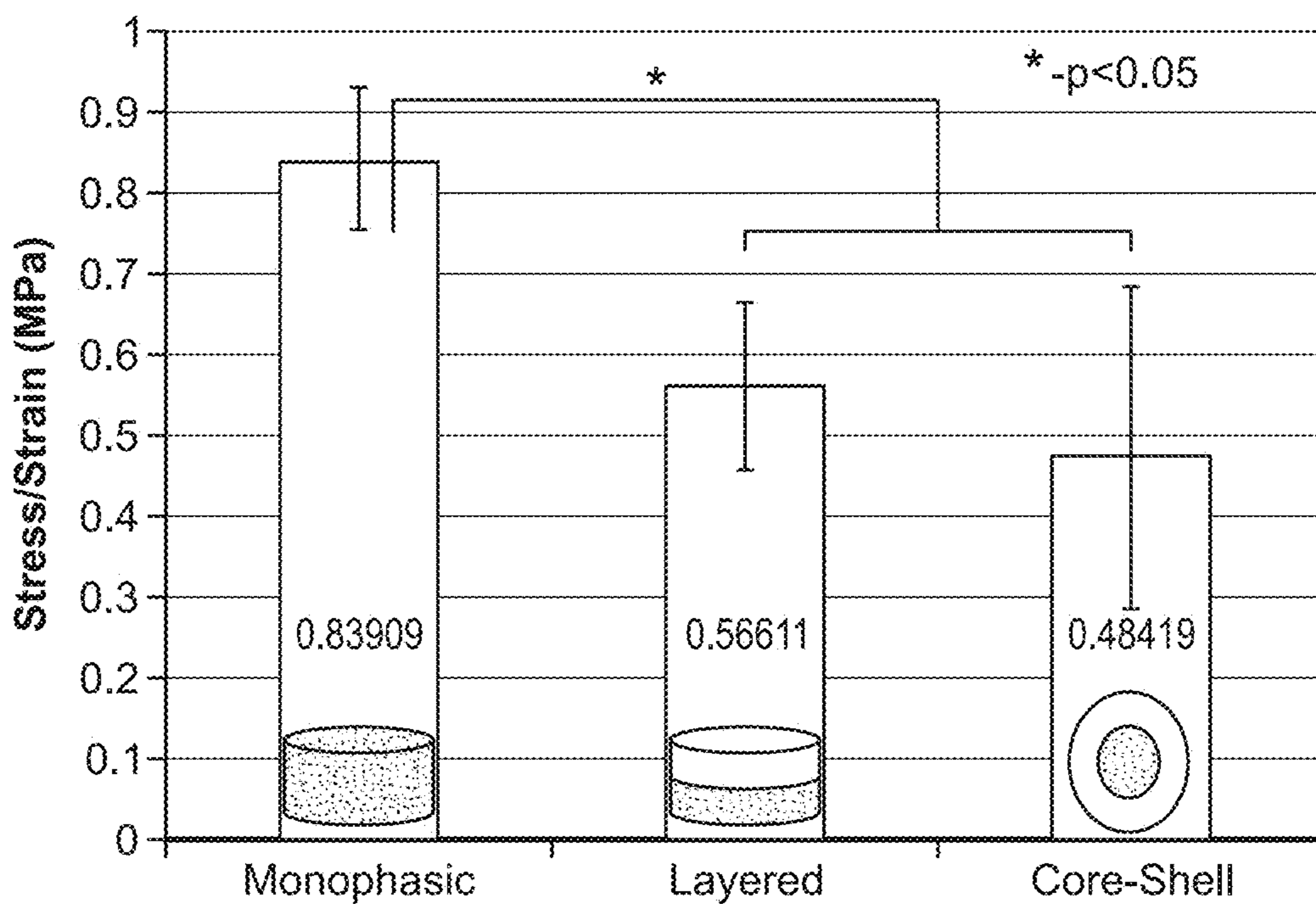


FIG. 21B

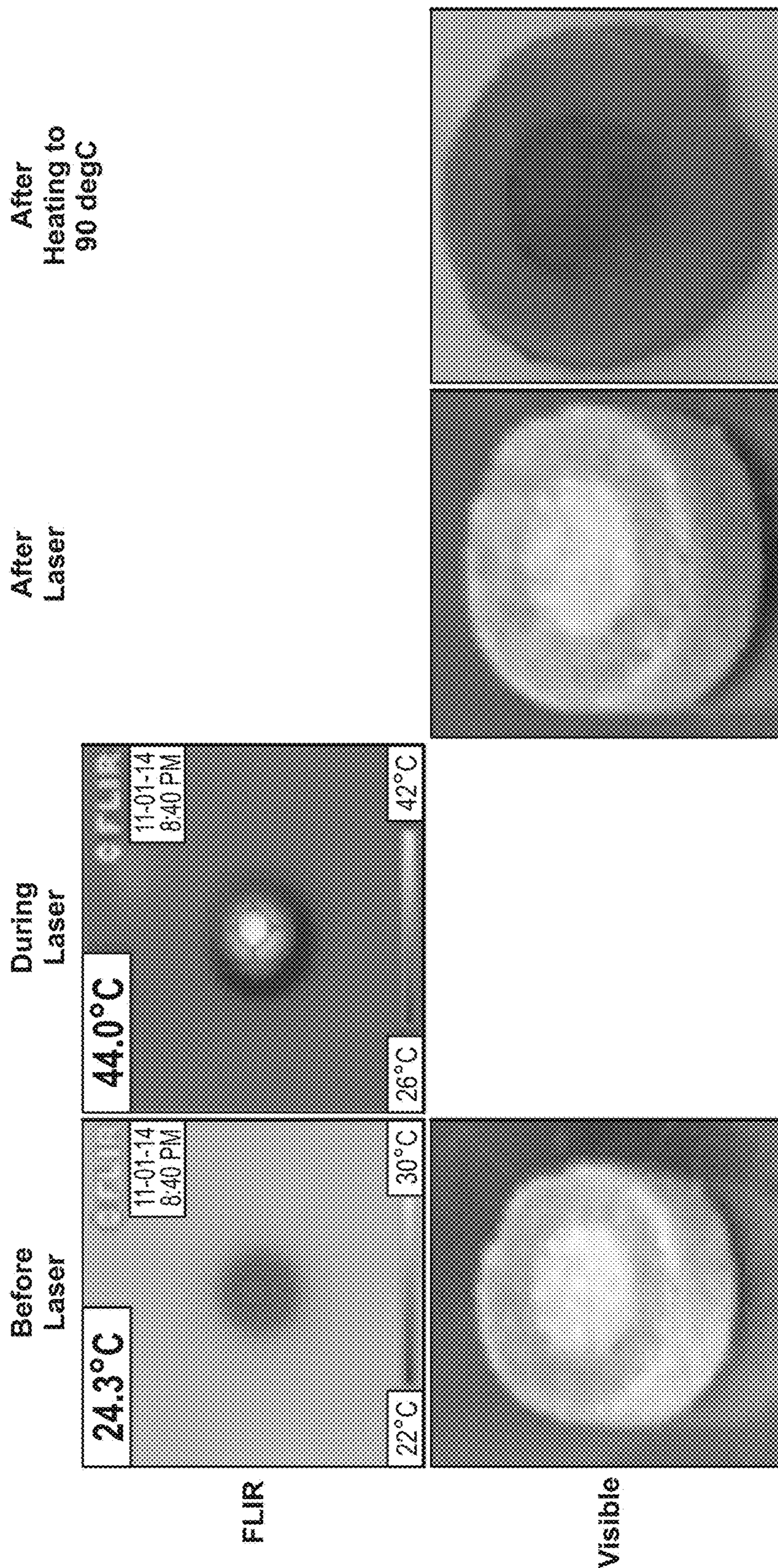


FIG. 21C

IN SITU ANTIGEN-GENERATING CANCER VACCINE

RELATED APPLICATIONS

This application is a continuation of U.S. Ser. No. 14/123, 615, which is a national stage application, filed under 35 U.S.C. § 371, of PCT International Application No. PCT/US2012/040687, filed Jun. 4, 2012, which claims the benefit of priority to U.S. Provisional Application No. 61/493,398, filed Jun. 3, 2011, the contents of each of which are incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

The invention relates generally to the field of cancer vaccines.

BACKGROUND OF THE INVENTION

Cancer accounts for approximately 13% of all human deaths worldwide each year. Existing dendritic cell-based therapeutic strategies are largely based on ex vivo manipulation of dendritic cells to generate large numbers of cells for activation with cancer antigen isolated from a biopsy of a patient's tumor. Because these ex vivo techniques are invasive and expensive, there is a pressing need to develop dendritic cell-based cancer vaccine strategies that are not dependent on surgical biopsies and ex vivo manipulation of cells.

SUMMARY OF THE INVENTION

The invention represents a significant breakthrough in the treatment of cancer in that tumor antigens for vaccination are generated without having to take a biopsy from the patient, process the tumor cells ex vivo, and then vaccinate the patient with processed tumor antigen. One can now achieve patient-specific cancer vaccines, without needing patient-specific manufacturing of the vaccine, via the generation of cancer antigens in situ using a scaffold that is implanted in the body. Live cancer cells are recruited to the 3-dimensional (3-D) vaccine scaffold following its placement in the patient, and the scaffold is treated to induce subsequent destruction of the recruited cancer cells. The destruction and/or lysis of the cancer cells generates antigens in situ in the scaffold.

Accordingly, the invention features a biopsy-free method for producing a processed (e.g., cell-dissociated) tumor antigen in situ. For example, the tumor antigen(s) are liberated from an intact tumor cell, associated with cell fragments, or associated with a cell that has been altered from its naturally-occurring state. First, a porous 3-dimensional scaffold is administered to a subject diagnosed with a cancer. The scaffold comprises a chemoattractant of cancer cells. Such molecules (and their amino acid (aa) and nucleic acid (na) sequences) are well known in the art. For example, the chemoattractant of cancer cells is a chemokine selected from the group consisting of chemokine (C-C motif) ligand 21 (CCL-21, GenBank Accession Number: (aa) CAG29322.1 (GI:47496599), (na) EF064765.1 (GI:117606581), incorporated herein by reference), chemokine (C-C motif) ligand 19 (CCL-19, GenBank Accession Number: (aa) CAG33149.1 (GI:48145853), (na) NM_006274.2 (GI:22165424), incorporated herein by reference), stromal cell-derived factor-1 (SDF-1, GenBank Accession Number, (aa) ABC69270.1 (GI:85067619), (na) E09669.1 (GI:

22026296), incorporated herein by reference), vascular endothelial growth factor (e.g., VEGFA; GenBank Accession Number: (aa) AAA35789.1 (GI:181971), (na) NM_001171630.1 (GI:284172472), incorporated herein by reference), and interleukin-4 (IL-4, GenBank Accession Number: (aa) AAH70123.1 (GI:47123367), incorporated herein by reference).

The scaffold is maintained in situ for a time period sufficient to accumulate circulating cancer cells, thereby yielding a cancer cell-containing scaffold. Finally, the cell-containing scaffold is contacted with a cytotoxic or cytolytic element to produce a processed tumor antigen. A cytotoxic or cytolytic element is a composition and/or condition that causes death or lysis, respectively, of a cell. For example, the cell is a cancer cell. The patient to be treated comprises a cancer that is characterized by circulating tumor cells, e.g., metastatic tumor cells. For example, the subject is diagnosed with a metastatic cancer condition or a blood-borne cancer or cancer of the circulatory system, e.g., leukemia. The cytotoxic or cytolytic element comprises a heat-conducting composition such as gold particles and/or the application of external heat, ultrasound, laser radiation, or gamma radiation. For example, cytotoxicity or cytolysis of a cancer cell is induced by applying a condition (e.g., an energy source such as those described above) to a cell-containing scaffold that also contains a heat-conducting composition. Suitable types of laser radiation include ultraviolet or near infrared laser radiation.

Exemplary scaffold compositions are described in U.S. 2008-0044900 A1 (incorporated herein by reference). Suitable scaffolds include polylactic acid, polyglycolic acid, co-polymers of polylactic acid and polyglycolic acid (e.g., PLGA polymers), alginates and alginate derivatives, gelatin, collagen, fibrin, hyaluronic acid, laminin rich gels, agarose, natural and synthetic polysaccharides, polyamino acids, polypeptides, polyesters, polyanhydrides, polyphosphazines, poly(vinyl alcohols), poly(alkylene oxides), poly(allylamines)(PAM), poly(acrylates), modified styrene polymers, pluronic polyols, polyoxamers, poly(uronic acids), poly(vinylpyrrolidone) and copolymers or graft copolymers of any of the above. One preferred scaffold composition includes an RGD-modified alginate.

The scaffold composition is between 0.01 mm³ and 100 mm³. For example, the scaffold composition is between 1 mm³ and 75 mm³, between 5 mm³ and 50 mm³, between 10 mm³ and 25 mm³. Preferably, the scaffold composition is between 1 mm³ and 10 mm³ in size.

The porosity of the scaffold influences ingress/egress of the cells from the device. Pores are nanoporous, microporous, or macroporous. The porous polymer device contains aligned and/or interconnected pores to facilitate movement of cells into and out of the device. For example, immune cells such as DCs are recruited into the device, pick up antigen, e.g., antigen that has been liberated from cancer cells that have been attracted to the device, and then migrate out of the device via the interconnected pores to leave the device and go to other sites in the body such as draining lymph nodes. For example, the diameter of nanopores are less than about 10 nm; micropores are in the range of about 100 nm-20 μm in diameter; and, macropores are greater than about 20 μm (preferably greater than about 100 μm, 200 μm, 300 μm and even greater than about 400 μm). In one example, the scaffold is macroporous with aligned or interconnected pores of about 400-500 μm in diameter.

Optionally, the scaffold further comprises a hyperthermia-inducing composition. Suitable hyperthermia-inducing compositions include a magnetic nanoparticle or a near infrared

(NIR) absorbing nanoparticle. In some cases, the nanoparticle is magnetic, and the method further comprises contacting the magnetic nanoparticle with an alternative magnetic field (AMF) to induce local hyperthermia in situ, thereby altering or disrupting the cancer cell and producing a processed tumor antigen. In another example, the method further comprises contacting the NIR nanoparticle with NIR radiation to induce local hyperthermia in situ, thereby altering or disrupting the cancer cell and producing a processed tumor antigen. Hyperthermia is characterized by a local temperature of greater than 37 degrees Celsius. For example, the temperature of the device is temporarily heated to 40, 45, 50, 60, 70, 75, 80, 85, 90, 95 or more degrees.

The size of the particles is tailored to the scaffolds of the invention. For example, the nanoparticle comprises a diameter of less than 200 nm, e.g., a diameter of greater than 2 nm and less than 150 nm, e.g., a diameter of 5-100 nm, e.g., a diameter of 10-50 nm. Exemplary particles are less than 45 nm, e.g., 40 nm, or less than 15 nm, e.g., 13 nm. A suitable NIR nanoparticle includes a gold nanorod, gold nanoshell, silica nanoparticle, gold nanocage, noble metal nanoparticle, carbon nanotube, carbon nanoparticle, and graphite nanoparticle.

The methods described herein are useful in the treatment of cancer in a mammal. The mammal can be, e.g., any mammal, e.g., a human, a primate, a mouse, a rat, a dog, a cat, a horse, as well as livestock or animals grown for food consumption, e.g., cattle, sheep, pigs, chickens, and goats. In a preferred embodiment, the mammal is a human.

The invention also provides a tumor antigen-processing device comprising a porous polymer, a chemoattractant for cancer cells, and a cell-altering or cell-destroying (e.g., cytotoxic or cytolytic) composition or element such as a hyperthermia-inducing particle. A hyperthermia-inducing nanoparticle is one that heats the cells within the scaffold to a cell-destructive temperature upon the application of an external energy source. For example, the energy source is a form of radiation such as heat, AMF or NIR.

An exemplary device comprises an immune cell (e.g., DC) recruitment composition such as granulocyte macrophage colony-stimulating factor (GM-CSF). In one example, the chemoattractant, cytotoxicity- or cytolysis-composition, and immune cell recruitment composition are interspersed throughout the porous polymer. In another example, the porous polymer comprises a first zone comprising the chemoattractant and cytotoxicity-inducing or cytolysis-inducing composition and a second zone comprising the immune cell recruitment composition. In the latter example, the zones are layered or constructed with a core-shell architecture, whereby the first zone is configured as a core and the second zone is configured as a shell. Exemplary cytotoxicity-inducing (or cytolysis-inducing) compositions are described above, e.g., hyperthermia-inducing particles.

As used herein, an "isolated" or "purified" nucleotide or polypeptide (e.g., a chemoattractant, cytokine, or chemokine nucleotide or polypeptide) is substantially free of other nucleotides and polypeptides. Purified nucleotides and polypeptides are also free of cellular material or other chemicals when chemically synthesized. Purified compounds are at least 60% by weight (dry weight) the compound of interest. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. For example, a purified nucleotides and polypeptides, e.g., a chemoattractant, cytokine, or chemokine is one that is at least 90%, 91%, 92%, 93%, 94%, 95%, 98%, 99%, or 100% (w/w) of the desired oligosaccharide by weight. Purity is measured by any appropriate

standard method, for example, by column chromatography, thin layer chromatography, or high-performance liquid chromatography (HPLC) analysis. The nucleotides and polypeptides are purified and used in a number of products for consumption by humans as well as animals, such as companion animals (dogs, cats) as well as livestock (bovine, equine, ovine, caprine, or porcine animals, as well as poultry). "Purified" also defines a degree of sterility that is safe for administration to a human subject, e.g., lacking infectious or toxic agents.

Similarly, by "substantially pure" is meant a nucleotide or polypeptide that has been separated from the components that naturally accompany it. Typically, the nucleotides and polypeptides are substantially pure when they are at least 60%, 70%, 80%, 90%, 95%, or even 99%, by weight, free from the proteins and naturally-occurring organic molecules with they are naturally associated.

Small molecules include, but are not limited to, peptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic and inorganic compounds (including heterorganic and organometallic compounds) having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 2,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

By the terms "effective amount" and "therapeutically effective amount" of a formulation or formulation component is meant a sufficient amount of the formulation or component to provide the desired effect. For example, "an effective amount" of a chemoattractant of cancer cells is an amount of a compound required to mediate an accumulation of two or more cancer cells in the scaffold device prior to application of a cell-altering or cell-destroying stimulus. Ultimately, the attending physician or veterinarian decides the appropriate amount and dosage regimen.

The terms "treating" and "treatment" as used herein refer to the administration of an agent or formulation to a clinically symptomatic individual afflicted with an adverse condition, disorder, or disease, so as to effect a reduction in severity and/or frequency of symptoms, eliminate the symptoms and/or their underlying cause, and/or facilitate improvement or remediation of damage. The terms "preventing" and "prevention" refer to the administration of an agent or composition to a clinically asymptomatic individual who is susceptible or predisposed to a particular adverse condition, disorder, or disease, and thus relates to the prevention of the occurrence of symptoms and/or their underlying cause.

The transitional term "comprising," which is synonymous with "including," "containing," or "characterized by," is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. By contrast, the transitional phrase "consisting of" excludes any element, step, or ingredient not specified in the claim. The transitional phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps and permits those that do not materially affect the basic and novel characteristic(s) of the claimed invention.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims. Unless otherwise defined, all technical and scientific terms used herein have

the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, Genbank/NCBI accession numbers, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a diagram of recruitment of peripheral dendritic cells (DCs) to the scaffold, loading of recruited DCs with cancer antigen, and DC maturation (induced by danger signals) such as CpG oligodeoxynucleotides (CpG-ODN)/poly(ethyleneimine) (PEI), e.g., PEI-condensed CpG ODN.

FIG. 2 is a diagram of circulating endogenous cancer cells being recruited to the vaccine scaffold following implantation in the patient, subsequent destruction of the recruited cancer cells leading to liberation of tumor antigens in situ in the scaffold, and DC activation/loading with liberated tumor antigen.

FIG. 3 is a bar graph showing migration of leukemia cells in response to a gradient of CCL-21.

FIG. 4 is a bar graph showing in vivo recruitment of leukemic cells to blank and loaded scaffolds.

FIG. 5 is a photograph of explanted scaffolds retrieved from mice showing fluorescent-nano particle (NP)-labeled leukemic cells that were recruited into the scaffold by CCL-21.

FIG. 6A is a photograph of a scaffold in which gold nanorods (GNRs) were incorporated in poly(lactide and glycolide) (PLG) macroporous scaffold (GNR-PLG scaffold).

FIG. 6B is a photomicrograph illustrating a microscopic view of the macroporous scaffold structure. The bar scale in the lower left-hand corner is 200 μm .

FIG. 6C is a photomicrograph showing gold nanorods (GNRs) coated with poly(ethylene glycol) (PEG).

FIG. 7A is a line graph showing the intrinsic maximum absorption of GNRs incorporated in the PLG scaffold.

FIG. 7B is a photomicrograph of a thermal image of a GNR-PLG scaffold after irradiation with 808 nm continuous diode laser.

FIG. 8A is a line graph demonstrating the temperature of GNR-PLG scaffolds after application of a different laser power.

FIG. 8B is a line graph demonstrating that repetitive near infrared (NIR) irradiation allowed multiple hyperthermia in the same GNR-PLG scaffold.

FIG. 9 is a photomicrograph of a thermal image of a GNR-PLG scaffold physically attached to a half blank PLG scaffold and irradiated with 808 nm laser.

FIG. 10A is a photograph of a single PLG scaffold composed of a GNR-incorporated core.

FIG. 10B is a photograph of a thermal image of a single PLG scaffold composed of a GNR-incorporated core.

FIG. 10C is a photomicrograph showing the well-interconnected pore structure of a PLG scaffold.

FIG. 11A is a bar chart illustrating heat shock protein (HSP) levels of cancer cell cultures after heat shock in 43° C.

FIG. 11B is a bar chart demonstrating in vitro DC activation by heat-shocked lysate and freeze-thaw lysate.

FIG. 12A is an illustration of cells (leukemic cells) in a GNR-PLG scaffold and irradiated with a laser (808 nm).

FIG. 12B is a bar chart showing the level of HSP70 in cancer cells after exposure to various temperatures.

FIG. 12C is a photograph of a western blot showing that 24 hour incubation after irradiation at 45° C. generated the highest HSP levels.

FIG. 13 is a bar chart representing the relative cell population in a GNR-PLG scaffold with activated DC cell surface markers after NIR irradiation at 45° C.

FIG. 14A is a photograph of a thermal image of an NIR irradiated GNR-PLG scaffold seeded with leukemic cells.

FIG. 14B is a bar chart showing leukemic cell viability in a GNR-PLG scaffold after application of NIR irradiation.

FIG. 15A is a photograph of a thermal image of an NIR irradiated GNR-PLG scaffold seeded with leukemic cells subcutaneously implanted in a C57BL/6J mouse.

FIG. 15B is a bar graph of a bar chart showing in vivo leukemic cell viability in a GNR-PLG scaffold after application of NIR irradiation.

FIGS. 16A-B are bar graphs showing DC recruitment and activation.

FIGS. 17A-B are bar graphs showing draining lymph node cell number and DC activation in draining lymph node tissue.

FIGS. 18A-D are bar graphs showing DC recruitment and activation in PLG vaccine using laser irradiation.

FIG. 19 is a bar graph showing DC activation with heat-shocked cancer cell lysate compared to freeze/thaw cancer cell lysate.

FIGS. 20A-B are scatter plots, and FIGS. 20C-D are bar graphs showing DC responsiveness to lipopolysaccharide (LPS).

FIG. 21A is a photograph of an antigen-generating cancer vaccine device with a core-shell architecture.

FIG. 21B is a bar graph showing the Young's modulus characteristics of an antigen-generating cancer vaccine with monophasic, layered, and core-shell architecture.

FIG. 21C is a series of photographs of an antigen-generating cancer vaccine scaffold device. The top panel shows the results of FLIR thermal imaging (thermography) of the device, and the bottom panel shows the results of visible imaging (photography).

DETAILED DESCRIPTION

Three dimensional (3D) scaffolds provide a temporary residence for dendritic cells (DCs) and effectively regulate host DC trafficking and activation in situ, while simultaneously preventing upregulation of the tolerizing arm of the immune system, and provide therapeutic protection against cancer. For example, in the cancer vaccine systems described herein, implantation of macroporous poly(lactide and glycolide) (PLG) scaffolds loaded with chemoattractant (GM-CSF) of DCs, cancer antigens (tumor lysates), and danger signals (CpG oligonucleotide) resulted in recruitment of peripheral DCs to the scaffold, loading of recruited DCs with cancer antigen, and their maturation by danger signals (FIG. 1). The antigen-presenting mature DCs moved to lymph nodes and generated potent cytotoxic T lymphocyte (CTL) responses. In this manner, this vaccine system triggered a strong anticancer immune response, which allowed the eradication of the cancer. One such system utilizes a tumor biopsy from the patient to be treated to generate the antigen, which requires ex vivo manipulation

and processing of tumor tissue. In addition, this system requires that each vaccine be manufactured for the specific individual to be treated (using the tumor lysate from that same patient). The methods of the present invention represent an improvement of the heretofore-described system.

As described herein, a patient-specific anti-tumor immune response and a reduction in tumor burden is achieved, without patient-specific manufacturing of the vaccine. Instead, cancer antigens are generated in situ in a polymeric scaffold that was implanted in the body. Live cancer cells present in the circulatory system of the subject are recruited to the vaccine scaffold following its placement in the patient, and the subsequent destruction of the recruited cancer cells generates antigens in situ in the scaffold (FIG. 2).

Recruitment of Circulating Cancer Cells to scaffolds

Suitable cancers to be treated in this manner are those in which there are circulating primary or metastatic cancer cells in the blood stream. These cells are characterized by migratory properties in response to gradients of specific chemokines. Thus, circulating cancer cells are recruited to implanted scaffolds in which specific chemokines have been incorporated.

Circulating cancer cells: metastatic cancer cells in various cancers, leukemic cells.

Chemoattractant of cancer cells: various chemokines depending on cancer type. (e.g., CCL-21, CCL-19, SDF-1, VEGF, IL-4 etc.).

3D scaffolds: various types of 3D scaffolds designed to have pores and to load chemokines including biodegradable porous polymer, porous inorganic materials, assembled nanoparticles, nanotubes, nanorods, etc.

Destruction of the Recruited Cancer Cells by External Stimuli on Scaffolds

As described below, in addition to recruitment of cancer cells to scaffolds, various external stimuli are used to kill the cancer cells after they are recruited in order to generate lysates containing cancer antigens in situ. External stimuli applicable to implanted scaffolds to kill the recruited cancer cells.

External Heating

Ultrasound

Laser irradiation: UV, Near infrared laser

Gamma irradiation

Nanoparticle (NP)-mediated hyperthermia

Alternative magnetic field (AMF) for scaffold loaded with magnetic nanoparticles.

Near infrared (NIR) irradiation for scaffold loaded with NIR absorbing nanoparticles (e.g., gold nanorods, gold nanoshells, gold nanocages, other noble metal nanoparticles, carbon nanotubes, carbon nanoparticles, graphite, etc.)

Separating the Manipulation of Recruited DCs and Cancer Cells

Sometimes it is desirable to separate the recruited DCs and cancer cells so the signals used to kill the cancer cells do not negatively impact the DC functions. As described below, this segregation is accomplished via control over the temporal order of recruitment of each cell type, or a spatial segregation of the cells that allows application of external stimuli to a specific region of scaffold.

Temporal Control of the Order of Cell Recruitment

Rather than recruiting all cells simultaneously, cancer cells are first recruited and destroyed to generate a lysate. Subsequently, cancer cells recruit DCs to the site of the cancer antigens without damaging DCs by external stimuli. For this purpose, it is possible to control of the release profiles from scaffolds of different chemoattractants to DCs and cancer cells. For example, different composition of

polymers with different degradation profiles and/or different molecular affinities to each chemokine are used in the preparation of a polymer vaccine scaffold to control release profiles.

Spatial Control of Scaffolds to Allow Applying External Stimuli to Specific Region of Scaffold

Scaffolds are also compartmentalized such that only certain compartments are affected by the external stimuli to specifically kill the cancer cells residing in those compartments, thereby allowing for the maintenance of intact and functional DCs in other compartments. For this purpose, various structural modifications of scaffolds are utilized. In the case of nanoparticle (NP)-mediated hyperthermia for killing of cancer cells, NPs are incorporated in specific regions of the scaffolds, which allows specific hyperthermia in the NP-region and has a trivial hyperthermic effect to other regions where DCs are recruited.

In Situ Antigen-Generating Cancer Vaccine

Immunotherapy with protein drugs (e.g., cytokines and monoclonal antibodies) is one approach for cancer management. Therapeutic cancer vaccines, another form of immunotherapy, represent another approach to treat cancer. Cancer vaccines are designed to invoke strong anti-tumor immune activity, and the induction of antigen-specific cytotoxic (CD8+) T lymphocytes (CTLs) is a critical aspect of their function. Activated CD8+ T cells kill tumor cells upon recognition of specific labels (antigens) present on tumor cells, and this recognition is dependent on binding of the label to a T cell receptor (TCR) specific to that antigen. Dendritic cells (DCs) are the most important antigen presenting cells (APCs), and play a key role in initiating CTL responses.

Prior to the invention described herein, the first DC-based therapeutic cancer vaccine, known as Provenge, was approved by the Food and Drug Administration. This breakthrough in cancer therapy demonstrated that the stimulation of a patient's own immune system to fight cancer. However, this therapy is based on ex vivo manipulation of DCs in order to generate large numbers of these cells, and to activate the cells with cancer antigen, and thus suffers from a high cost and significant regulatory burden. In addition, tumors were not eradicated with this therapy, and the increase in patient survival time has been limited to 4 months. While this breakthrough may have a major impact on cancer treatment, it also highlights the need to make further progress on the DC-based cancer vaccine strategy, and to bypass its dependency on ex vivo manipulation.

Developments in material science have led to new biomaterials and the applications of materials in a wide range of biomedical applications, including diagnostics, cancer therapy, and tissue regeneration. In particular, nanoparticles and macroscopic, three-dimensional biomaterials have significant potential in many clinical applications. As described herein, because of their nanosize and easy surface modification, targeting of nanoparticles to various tissues, including tumors and lymph nodes is exploited to deliver imaging or therapeutic modalities. 3-D macroscale biomaterials, especially porous scaffolds, have been extensively explored for applications involving the controlled release of growth factors, cell delivery, and tissue regeneration. These materials create microenvironments that allow the fate of resident cells to be modulated, typically via control over the physical properties and presentation of cell signaling molecules from the walls of the materials. These 3-D macroscale materials and nanoparticles are useful in the development of vaccines in the context of cancer, particularly via the targeting and programming of specific immune cell populations.

As described in the examples below, porous polymer matrices that provide a temporary residence for DCs effectively regulate host DC trafficking and activation in situ, while simultaneously preventing upregulation of the tolerizing arm of the immune system, and provide therapeutic protection against cancer. Macroporous PLG scaffolds incorporating i) GM-CSF to recruit DCs, ii) CpG/PEI complex to mature the DCs, and iii) tumor lysate to provide a mixture of cancer antigens were developed for this purpose. Upon subcutaneous implantation, GM-CSF was released and established a gradient in the surrounding tissue to recruit significant numbers of host DCs. The presentation of CpG/PEI complex from the polymer to the recruited DCs increased the maturation of DCs in the scaffolds and their LN-homing. These scaffolds induced strong specific CTL responses to melanoma in a prophylactic model, with a 90% survival rate as well as in therapeutic models of melanoma and glioblastoma with over a 50% survival rate after vaccinations. This system recruited various DC subsets, including significant numbers of plasmacytoid DCs (pDCs) and CD8+ DCs, which are very important in antigen cross presentation, and the numbers of these DC subsets strongly correlated with the vaccine efficacy. This vaccine also diminished the local concentrations of tolerogenic cytokines (e.g., IL-10, TGF- β), and numbers of T regulatory cells, suggesting that a key aspect of its success related to its ability to down-regulate tolerance. These effects were only found when the polymer had the physical form of a macroporous scaffold, as the vaccine effectiveness was significantly diminished when polymer microspheres were used instead to provide a sustained, localized release of the bioactive agents, without providing a residence for the recruited cells. This result indicates that creating a microenvironment in which host environmental cues are minimized, and exogenous maturation factors are highly concentrated, is a key to reprogram immune responses in situations such as cancer where there exist significant, pathology-associated tolerizing cues.

However, a limitation in this system is that it requires a tumor biopsy from patients and ex vivo manipulation and processing to generate cancer antigens. A system to generate cancer antigens in situ in the scaffold implanted in the body without biopsy or any ex vivo manipulation of cells represents an improvement over earlier systems. To make a patient-specific cancer vaccine, an improved scaffold system was developed in which cancer cells are recruited to a 3D vaccine scaffold and the alteration or destruction of those recruited cancer cells generates cell lysates in situ in the scaffold.

Example 1: In Situ Antigen-Generating Cancer Vaccine by Recruiting Cancer Cells and Subsequent Destruction of the Recruited Cancer Cells by External Stimuli

Described below are examples of gold nanorod-loaded cancer vaccine scaffolds to recruit leukemic cells and their subsequent destruction via NIR irradiation-mediated hyperthermia to generate cancer antigen coupled with heat shock protein. To demonstrate the scaffolds are capable of recruiting cancer cells, mouse leukemic cells (C1498) were tested in transwell assay using CCL-21 as the chemoattractant. C1498 showed strong migration to the gradients of CCL-21 (FIG. 3).

Example 2: In Vivo Recruitment of Leukemic Cells

In vivo recruitment of leukemic cells was characterized using GFP-expressing leukemic cells. PLG scaffolds with-

out any chemokines (Blank), loaded with GM-CSF (G), and loaded with GM-CSF and CCL-21(G+C) were implanted to C57BL/6J mice subcutaneously and GFP-leukemic cells were injected into blood via tail vein injection at Day 4. The scaffolds were retrieved at Day 6 and the cells in scaffolds were isolated and analyzed in FACS (FIG. 4). In addition, the scaffolds retrieved from mice injected with fluorescent-NP-labeled leukemic cells were imaged under fluorescent imaging instrument (Xenogel) (FIG. 5). Both results presented that CCL-21 released from scaffold increased the recruitment of leukemic cells in the animal.

Example 3: Hyperthermia-Mediated Antigen Generation

To achieve hyperthermia-mediated antigen generation from recruited cancer cells, gold nanorods (GNRs) were incorporated in PLG macroporous scaffold (GNR-PLG scaffold) during the fabrication step (FIG. 6). GNR-PLG scaffold had 250-440 μm pores and GNRs were incorporated over the whole scaffold, resulting in dark color in the resulting PLG scaffold. The surface of GNRs were modified with poly(ethylene glycol) (PEG) to remove the toxicity from original surfactants (cetyltrimethylammonium bromide) used in GNR-fabrication step which is known as toxic agents to the cells.

GNRs incorporated in PLG scaffold showed intrinsic maximum absorption at ~ 810 nm with maximum intensity, which is desirable for in vivo irradiation due to minimum absorption by tissue and water in that range of wavelength (FIG. 7A). Upon irradiation with 808 nm continuous diode laser, the temperature of GNR-PLG scaffold was increased to 40° C. (FIG. 7B) from room temperature.

As described below, the temperature of GNR-PLG scaffolds were controlled in the range from room temperature up to $\sim 70^\circ$ C. by applying different power of laser (FIG. 8A). By contrast, blank scaffold without incorporation of GNRs showed insignificant change in temperature upon irradiation with even highest power that was applied to GNR-PLG scaffold, representing the NIR-mediated hyperthermia could be induced specifically in GNR-incorporated scaffold. Optionally, multiple antigen generation is used to elicit a strong immune activation. The repetitive NIR irradiation allowed multiple hyperthermia in same GNR-PLG scaffold without decrease of the targeting temperature by using same laser power (FIG. 8B).

Example 4: GNR Absorption of NIR Light

Local hyperthermia for GNR-incorporated region of PLG scaffold was possible due to the absorption of NIR light by GNRs in specific area of PLG scaffold. A half GNR-PLG scaffold and a half blank PLG scaffold were physically attached and irradiated with 808 nm laser with large beam size to cover whole scaffold, resulting in specific heating in only GNR-PLG scaffold side (FIG. 9). This configuration represents a scaffold with different compartments, i.e., one for cancer cells and one for DCs that can be implanted. Only the compartment for cancer cells is heated, while the one for DCs remains intact (unheated) to allow normal DC function.

Example 5: A PLG Scaffold with a GNR-Incorporated Core and a Normal Shell

A single PLG scaffold composed of GNR-incorporated core part and normal shell part (FIG. 10A) was fabricated for cancer-specific heating (FIG. 10B). This single PLG scaffold

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with different compartment and well-interconnected pore structure (FIG. 10C) allows the efficient cancer antigen uptake by DC after hyperthermia (FIG. 10C).

Example 6: Heat-Shocked Cancer Cells

To test if heat-shock can induce more immunogenic antigens due to adjuvant effect of HSP produced from heat-shocked cancer cells, HSP levels of cancer cell cultures were analyzed after heat shock in 43° C. water bath (FIG. 11A). Higher HSP70, a representative HSP, were obtained both the cell lysate and cell culture media in heat-shocked condition compared with freeze-thaw method, the common lysate generating method. In vitro DC-activation by heat-shocked lysate and freeze-thaw lysate showed that heat-shocked lysate have higher DC-activating property due to higher HSPs (FIG. 11B).

Example 7: NIR-Irradiation to Induce HSPs from Cancer Cells

To test if NIR-irradiation on GNR-PLG scaffold can induce HSPs from cancer cells residing in scaffold, leukemic cells were seeded in GNR-PLG scaffold and subsequently irradiated with 808 nm laser (FIG. 12A). The various temperatures by irradiation were tested for to evaluate HSP70 levels, representing that irradiating to 45° C. resulted maximum HSP70 level (FIG. 12B). In addition, Western blot data showed that 24 hour incubation after irradiation at 45° C. generated highest HSPs.

Example 8: Activation of Dendritic Cells

In vitro DC, e.g., bone-marrow derived dendritic cells (BMDC), activation with cell lysates from GNR-PLG scaffold after NIR irradiation at 45° C. resulted in higher activation of DCs compared with non-irradiated cell lysates in terms of CCR7 and CD86, the representative cell surface markers of activated DCs (FIG. 13), representing NIR irradiation leads to in situ generation of highly immunogenic cancer antigens from cancer cells residing in GNR-PLG scaffold.

Example 9: Cancer Cell Viability after NIR Irradiation In Vitro

In vitro cancer cell viability in GNR-PLG scaffold after NIR irradiation was evaluated. Leukemic cells were seeded in GNR-PLG scaffold and NIR irradiation was applied to increase temperature to 40, 45, and 50° C., and the viability of cells was checked with Alamar blue assay (FIG. 14). Lower cell viability resulted from higher temperature. In addition, a second irradiation resulted in even lower viability in all conditions. These data indicate that the cancer cells were dying due to hyperthermia caused by NIR irradiation in GNR-PLG scaffold.

Example 10: Cancer Cell Viability after NIR Irradiation in Vivo

To mimic in vivo recruitment, the GNR-PLG scaffold seeded with leukemic cells were implanted into the tissues of C57BL/6J mice subcutaneously, and the scaffolds were irradiated with NIR laser to 45 and 50° C. Similar to the in vitro experiments, the cell viability was decreased in conditions of higher temperatures (FIG. 15), indicating that the NIR-irradiation induced heat-shock to the recruited cancer

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cells in GNR-PLG scaffold. Recruitment of circulating cancer cells into the implanted scaffold device, alteration or destruction of the cancer cells by application of an external force, e.g., radiation, leads to increased availability of tumor antigens in the device. The increased availability of tumor antigens in the device for cancer leads to an increase in DC activation and to a more effective cancer vaccine.

Example 11: In Vivo Irradiation of Implanted Device Leads to Increase in DC Activation and Number of Recruited DC's

In vivo dendritic cell recruitment and activation using laser irradiation were evaluated. 10⁶ EG7.Ova lymphoma cells were loaded into GNR-PLG scaffolds with GM-CSF. The scaffolds were implanted subcutaneously in C57BL/6J mouse. On day 3 post implantation the scaffold site was irradiated with 808 nm NIR laser to 45° C. for 5 minutes. On day 7 post implantation, the scaffolds were retrieved, digested, and the cells were analyzed for the dendritic marker (CD11c), and the activation marker (CD86). FIGS. 16A-B show that laser irradiation significantly (n=3, p<0.05) increases the percentage of recruited dendritic cells (A) activates them in the scaffold (B).

Example 12: In Vivo Irradiation of Implanted Device Leads to Increase in DC Activation and Number of Activated DCs in Draining Lymph Node

In vivo dendritic cell activation in the draining lymph node using laser irradiation was evaluated. 10⁶ EG7.Ova lymphoma cells were loaded into GNR-PLG scaffolds with GM-CSF. The scaffolds were implanted subcutaneously in the back of a C57BL/6J mouse. On day 3 post implantation the scaffold site was irradiated with 808 nm NIR laser to 45° C. for 5 minutes. On day 7 post implantation, the draining lymph nodes (inguinal lymph nodes) were retrieved, digested, and the cells were analyzed for the dendritic marker (CD11c), and the activation marker (CD86). FIGS. 17A-B show that laser irradiation significantly (n=3, p<0.05) enlarges the draining lymph node (A), which is a response after inflammation, and increases the number of activated dendritic cells (B) in the lymph node. These data indicate that recruited DCs leave the scaffold device and migrate/relocated to draining lymph nodes (i.e., an anatomical site different from the location of the implanted device).

Example 13: Irradiation as an Additional Danger Signal for Immune Cell Activation

In vivo dendritic cell recruitment and activation in the full PLG vaccine using laser irradiation were evaluated. 10⁶ EG7.Ova lymphoma cells were loaded into GNR-PLG scaffolds with GM-CSF and condensed CpG-ODN, which serves as the danger signal to activate DCs. The scaffolds were implanted subcutaneously in C57BL/6J mouse. On day 3 post implantation the scaffold site was irradiated to 45° C. for 5 minutes with 808 nm NIR laser. On day 7 post implantation, the scaffolds were retrieved, digested, and the cells were analyzed for the dendritic marker (CD11c), and the activation marker (CD86). FIGS. 18A-D show that laser irradiation further increases (n=3, p<0.05) the percentage (A) and total number (B) of recruited DCs, and activates them at the scaffold site (C-D). This data indicate indicates that laser irradiation serves as an additional danger signal for immune cell activation.

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Example 14: Hyperthermic Treatment of Cancer Cells

In vitro BMDC activation with heat shock B16 cell lysate was evaluated. 10×10^6 B16 melanoma cells were heat shocked at 45°C . for 5 minutes in pre-warmed water bath to prepare heat-shocked cell lysate. Conventional cell lysate was generated using 3 cycles of freeze-thaw procedure. The prepared lysate was incubated with 10^6 BMDCs for 18 hours. BMDC activation markers, MHCII and CD86, were analyzed using flow cytometry. Cells were gated on CD11c+ DCs. FIG. 19 shows that the lysate generated from heat shocked cells serves as a danger signal to activate DCs, because it is capable of generating more ($p < 0.05$) activated DCs than conventional cell lysates.

Example 15: Effect of Temperature on LPS Responsiveness

High temperature induces reduced responsiveness to LPS in BMDC in vitro. 10^6 /well BMDCs were heat shocked at 50°C . for 5 minutes. They were then incubated with or without LPS, an immune adjuvant capable of upregulating immune cell activation. Activation markers, CD86 and MHC-II, were analyzed in flow cytometry. FIGS. 20 A-D show that heat shock can abrogate the BMDCs' capability to respond to LPS stimulation. Thus, an alternative scaffold structure was developed to protect recruited DCs from irradiation.

Example 16: Device with Core-Shell Architecture

An alternative structure of GNR scaffold, a core-shell type scaffold, was engineered to avoid the direct killing of recruited BMDCs but to allow for the heat shock of recruited cancer cells. The inner core scaffold is designed to load cancer recruiting chemokine and GNR (the color is dark due to loaded GNR); the outer shell scaffold is loaded with only GM-CSF to recruit DCs. Compressive testing demonstrates that this scaffold scheme has a lower Young's modulus than the conventional scaffold scheme (FIGS. 21A-C). In this design, only the cancer cells that are recruited to the inner core scaffold are subjected to heat shock from laser irradiation and the recruited DCs in outer shell scaffold avoid heat shock.

Other Embodiments

While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All United States patents and published or unpublished United States patent applications cited herein are incorporated by reference. All published foreign patents and patent applications cited herein are hereby incorporated by reference. Genbank and NCBI submissions indicated by accession number cited herein are hereby incorporated by reference. All other published references, documents, manuscripts and scientific literature cited herein are hereby incorporated by reference.

While this invention has been particularly shown and described with references to preferred embodiments thereof,

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it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

The invention claimed is:

1. A biopsy-free method for producing a processed tumor antigen in situ comprising administering to a subject having a cancer comprising circulating tumor cells a porous 3-dimensional scaffold that does not comprise a tumor antigen before the scaffold is administered to the subject, said scaffold comprising
 - (a) a chemoattractant for a cancer cell, wherein the chemoattractant for the cancer cell is released from the scaffold with a first release profile; and
 - (b) an immune cell recruitment composition, wherein the immune cell recruitment composition is released from the scaffold with a second release profile, such that cancer cells are recruited to the scaffold prior to recruitment of immune cells;
 maintaining the scaffold in situ for a period of time sufficient to accumulate circulating cancer cells, to yield a cancer cell-containing scaffold; and
 - contacting said cancer cell-containing scaffold with a cytotoxic or cytolytic element to produce a processed tumor antigen, wherein the scaffold recruits an immune cell to the site of the processed tumor antigen after the processed tumor antigen is produced.
2. The method of claim 1, wherein said cytotoxic element comprises application of external heat, ultrasound, laser radiation, or gamma radiation to said cell-containing scaffold.
3. The method of claim 1, wherein said scaffold further comprises a hyperthermia-inducing composition.
4. A polymer vaccine scaffold device that first recruits a cancer cell that is destroyed to generate a lysate, and subsequently recruits a dendritic cell, wherein the device comprises
 - (a) a cytotoxicity-inducing composition,
 - (b) a chemoattractant for a cancer cell, wherein the chemoattractant for the cancer cell is released from the scaffold with a first release profile; and
 - (c) an immune cell recruitment composition, wherein the immune cell recruitment composition is released from the scaffold with a second release profile, such that cancer cells are recruited to the scaffold prior to recruitment of immune cells following administration of the device to a subject;
 wherein said device does not comprise a tumor antigen before said device is administered to the subject.
5. A biopsy-free method for producing a processed tumor antigen in situ comprising administering to a subject diagnosed with a cancer the device of claim 4, thereby producing a processed tumor antigen in situ in the subject.
6. A method of treating cancer in a subject in need thereof, comprising administering the device of claim 4 to the subject, thereby treating cancer in the subject.
7. A method of activating an immune cell in a subject in need thereof, comprising administering the device of claim 4 to the subject and, after a period of time sufficient for the device to accumulate circulating cancer cells, irradiating the site of administration with a laser.
8. The method of claim 7, wherein said immune cell comprises a dendritic cell.
9. The method of claim 7, wherein the device is administered by implantation into said subject.

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10. The method of claim 9, wherein said implantation comprises subcutaneous implantation.

11. The method of claim 1, wherein the immune cell comprises a dendritic cell.

12. The method of claim 1, wherein the chemoattractant for a cancer cell is a chemokine.

13. The method of claim 12, wherein the chemoattractant for a cancer cell is selected from the group consisting of CCL-21, CCL-19, SDF-1, VEGF, and IL-4.

14. The method of claim 12, wherein the chemoattractant for a cancer cell is CCL-21.

15. The method of claim 1, wherein the immune cell recruitment composition comprises GM-CSF.

16. The method of claim 1, wherein the scaffold further comprises CpG oligodeoxynucleotides (CpG-ODN).

17. The method of claim 3, wherein the hyperthermia-inducing composition comprises a magnetic nanoparticle or a near infrared (NIR) absorbing nanoparticle.

18. The method of claim 17, wherein the hyperthermia-inducing composition comprises a magnetic nanoparticle, and wherein said cytotoxic or cytolytic element comprises an alternative magnetic field.

19. The method of claim 17, wherein the hyperthermia-inducing composition comprises a NIR absorbing nanoparticle, and wherein said cytotoxic or cytolytic element comprises NIR radiation.

20. The method of claim 17, wherein the hyperthermia-inducing composition comprises a NIR absorbing nanoparticle selected from the group consisting of a gold nanorod, a gold nanoshell, a gold nanocage, a noble metal nanoparticle, a carbon nanotube, a carbon nanoparticle, and a graphite nanoparticle.

21. The method of claim 20, wherein the hyperthermia-inducing composition comprises a gold nanorod or a gold nanoshell.

22. The method of claim 1, wherein the scaffold comprises a first polymer comprising the chemoattractant, and a second polymer comprising the immune cell recruitment composition.

23. The method of claim 22, wherein the first polymer has a different degradation profile than the second polymer.

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24. The method of claim 1, wherein the cancer comprises metastatic cancer cells.

25. The method of claim 1, wherein the cancer is a blood-borne cancer.

26. The method of claim 25, wherein the cancer is leukemia.

27. The method of claim 1, wherein the subject is a human subject.

28. The device of claim 4, wherein the chemoattractant for a cancer cell is a chemokine.

29. The device of claim 4, wherein the chemoattractant for a cancer cell is selected from the group consisting of CCL-21, CCL-19, SDF-1, VEGF, and IL-4.

30. The device of claim 4, wherein the chemoattractant for a cancer cell is CCL-21.

31. The device of claim 4, wherein the immune cell recruitment composition comprises GM-CSF.

32. The device of claim 4, wherein the second polymer composition further comprises CpG oligodeoxynucleotides (CpG-ODN).

33. The device of claim 4, wherein the cytotoxicity-inducing composition comprises a hyperthermia-inducing particle.

34. The device of claim 33, wherein the wherein the hyperthermia-inducing particle comprises a magnetic nanoparticle or a near infrared (NIR) absorbing nanoparticle.

35. The device of claim 33, wherein the hyperthermia-inducing particle comprises a NIR absorbing nanoparticle selected from the group consisting of a gold nanorod, a gold nanoshell, a gold nanocage, a noble metal nanoparticle, a carbon nanotube, a carbon nanoparticle, and a graphite nanoparticle.

36. The device of claim 33, wherein the hyperthermia-inducing particle comprises a gold nanoparticle or a gold nanorod.

37. The device of claim 4, wherein the device comprises a first polymer comprising the chemoattractant, and a second polymer comprising the immune cell recruitment composition.

38. The device of claim 37, wherein the first polymer has a different degradation profile than the second polymer.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 10,406,216 B2
APPLICATION NO. : 15/345131
DATED : September 10, 2019
INVENTOR(S) : Jaeyun Kim et al.

Page 1 of 2

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims

In Claim 32 (Column 16, Line numbers 17-19), delete “The device of claim 4, wherein the second polymer composition further comprises CpG oligodeoxynucleotides (CpG-ODN)” and replace it with “The device of claim 4, wherein the cytotoxicity-inducing composition comprises a hyperthermia-inducing particle”.

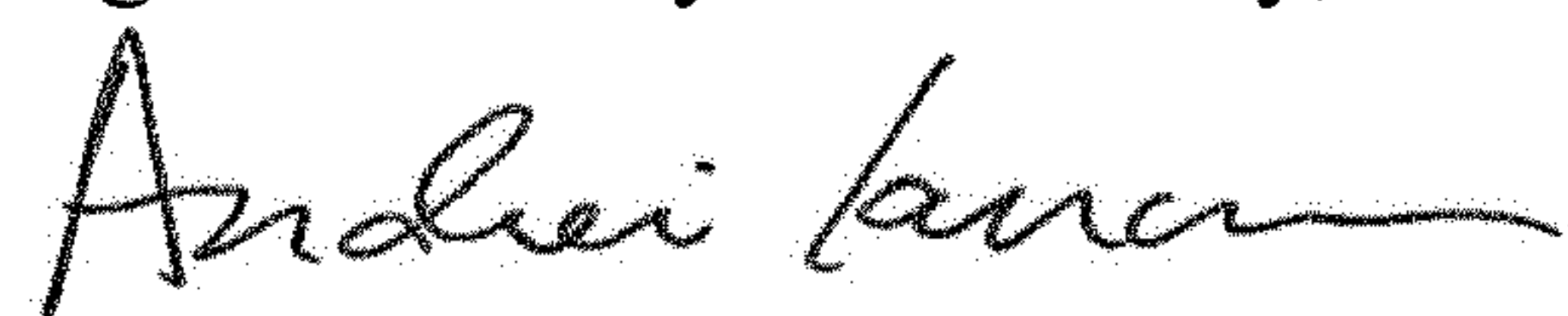
In Claim 33 (Column 16, Line numbers 20-22), delete “The device of claim 4, wherein the cytotoxicity-inducing composition comprises a hyperthermia-inducing particle” and replace it with “The device of claim 32, wherein the hyperthermia-inducing particle comprises a magnetic nanoparticle or a near infrared (NIR) absorbing nanoparticle”.

In Claim 34 (Column 16, Line numbers 23-25), delete “The device of claim 33, wherein the wherein the hyperthermia-inducing particle comprises a magnetic nanoparticle or a near infrared (NIR) absorbing nanoparticle” and replace it with “The device of claim 32, wherein the hyperthermia-inducing particle comprises a NIR absorbing nanoparticle selected from the group consisting of a gold nanorod, a gold nanoshell, a gold nanocage, a noble metal nanoparticle, a carbon nanotube, a carbon nanoparticle, and a graphite nanoparticle”.

In Claim 35 (Column 16, Line numbers 26-31), delete “The device of claim 33, wherein the hyperthermia-inducing particle comprises a NIR absorbing nanoparticle selected from the group consisting of a gold nanorod, a gold nanoshell, a gold nanocage, a noble metal nanoparticle, a carbon nanotube, a carbon nanoparticle, and a graphite nanoparticle” and replace it with “The device of claim 32, wherein the hyperthermia-inducing particle comprises a gold nanoparticle or a gold nanorod”.

In Claim 36 (Column 16, Line numbers 32-34), delete “The device of claim 33, wherein the hyperthermia-inducing particle comprises a gold nanoparticle or a gold nanorod” and replace it with “The device of claim 4, wherein the device comprises a first polymer comprising the chemoattractant, and a second polymer comprising the immune cell recruitment composition”.

Signed and Sealed this
Eighteenth Day of February, 2020



Andrei Iancu
Director of the United States Patent and Trademark Office

In Claim 37 (Column 16, Line numbers 35-38), delete “The device of claim 4, wherein the device comprises a first polymer comprising the chemoattractant, and a second polymer comprising the immune cell recruitment composition” and replace it with “The device of claim 36, wherein the first polymer has a different degradation profile than the second polymer”.

In Claim 38 (Column 16, Line numbers 39-40), delete “The device of claim 37, wherein the first polymer has a different degradation profile than the second polymer.” and replace it with “The device of claim 36, wherein the second polymer further comprises CpG oligodeoxynucleotides (CpG-ODN)”.